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The etiology of tulip fire.

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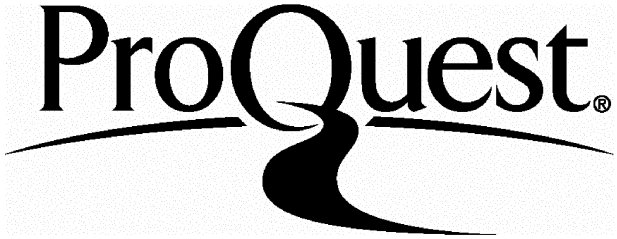


THE ETIOLOGY OF TULIP FIRE

by

D. Price

Summary

"Primaries" are traditionally defined as bulbs with foliage already infected when emerging above ground, but the investigation showed that viable propagules of B. tulipae represent the true primary phase of tulip fire disease; traditional primaries reflecting the spread of B. tulipae conidia from primary bulb-borne sources.

Bulb development after planting was relatively insensitive to changing temperatures which, however, affected the development of B. tulipae. At 15.5°C inocula of B. tulipae carried on bulbs spread rapidly infecting foliage and sometimes killing the flowering shoot before emergence above ground. At lower temperatures, B. tulipae invaded the subterranean bases of flowering shoots causing changes reflected in shorter brittle shoots with paler green foliage. At 4°C, B. tulipae spread slowly invading developing daughter bulbs without affecting the longevity of the mother plant. Two distinct pathways of spread from mother to daughter bulbs were identified, an inner and an outer.

Conidia produced on foliage already infected when emerging above ground were involved in the secondary aerial spread of B. tulipae. Aerially dispersed conidia caused either aggressive or non-aggressive lesions, the former, which ultimately sporulate, being favoured by (a) increasing numbers of conidia/infection drop and (b) prolonged humid periods.

Minimising the incidence of foliage lesions, caused by aerially spread conidia, increased bulb yields. Of the fungicides applied at two weekly intervals, mancozeb and dichlofluanid formulations gave the best control. Yields were sometimes increased by the same range of fungicides in the absence of B. tulipae, possibly by decreasing the damaging lipolytic activity of 'saprophytic' colonisers of the tulip phylloplane.

TABLE OF CONTENTS

PLATES

INTRODUCTION

Although the origin of many cultivated tulips is unknown, Hall (1940) considered that most were derived from *Tulipa gesneriana* L., an indeterminate species occurring naturally from the Caucasus to Eastern Europe. Tulips were probably imported into Britain *c.* 1578, the first description of the flower having been published in English at that time (Lyte, 1578). Since then interest has steadily increased (*vide* Mackay, 1852), with a total of 3700 acres being planted in 1966, mostly in East Anglia. Of this area, 60% was devoted to the production of dry and forced bulbs, the remainder being used for flower production out-of-doors. The acreage planted for the production of dry and forced bulbs has increased by a factor of x 1.8 in the last fifteen years and foreign exchange restrictions are likely to lead to further increases at the expense of imports. Dry bulbs are those replanted to maintain or increase stocks, flowers being removed once their trueness to type has been established. Bulbs used for forcing are those exposed to artificial cycles of warm and cool conditions in order to hasten and permit out-of-season flowering.

Tulips are commonly grown in 'field beds' or on a ridge system. In the former, 9 - 10 cm diameter bulbs are manually planted at a rate of 200,00 per acre in rows spaced 10 in apart with every seventh or eighth row remaining vacant. However, with increasing labour costs and the availability of less damaging machinery, a greater proportion of bulbs is now machine-planted in a density of about 160,00 per acre. With better designed lifting machinery becoming available there is a trend for ridges to be widened, i.e. , to a semi-ridge system of planting, to

include more rows per ridge so increasing numbers of bulbs per acre.

To succeed as a pathogen, Botrytis tulipae (Lib.) Lind., has to be superimposed upon the pattern of activity of the tulip bulb and to understand the disease presupposes an understanding of the host and its own life cycle.

Until comparatively recently it was generally assumed that tulip bulbs were dormant in the interval between leaf senescence and the onset of shoot growth in the ensuing winter. Physiological studies have shown that this assumption is far from the truth. A tulip bulb is an ovoid conical bud with a rounded base, termed a root plate, covered with a tough brownish, membraneous scale, the tunic. Beneath the tunic is the outer fleshy scale and near the root plate there may be a small bulb. Further dissection of the bulb reveals that it is composed of a series of concentric fleshy scales and in each axil there is one or more buds. The influence of the growing season on bulbs is considerable and the degree of development at lifting will vary from one season to another. If bulbs are examined at lifting, i.e. in summer when the leaves have senesced, the main central bulb is usually small but will gradually differentiate during storage until late August when all the floral parts within the furled leaves may be discerned. Small bulbs, usually those under 8 - 9 cm diameter, will not possess a flower bud and only one leaf will have formed - these small flowerless bulbs are termed 'maidens'. Warm storage favours differentiation and subsequent cooler conditions, after differentiation, permit extension growth. At lifting, bulbs for normal seasonal flower production are stored at 24°C and gradually cooled to 17°C by the end of October. It is the manipulation of these storage temperatures that permits out-of-season flower

production. Bulbs planted at the correct time will produce roots before shoots elongate. If planting is delayed shoots elongate in store, suggesting that root and shoot development are independent.

B. tulipae, the cause of fire, is the most troublesome fungal pathogen of tulips. It was first recorded in the Ardennes in 1830 (Saccardo, 1888-89), but authenticated accounts of the disease were not published until 1888 (Cavara, 1888 a, b). B. tulipae causes leaf and flower spotting, lesions sometimes coalescing with the subsequent collapse of the host. Tulip fire was first reported in Britain in 1899 (Massee, 1899), although the fungus had probably been isolated earlier as Moore (1939) considered that Polyactis cana was incorrectly identified by Berkeley in 1884. Furthermore, there is little doubt that other early instances were incorrectly ascribed to Sclerotium tuliparum Kleb. Beaumont, Dillon Weston and Wallace (1936) noted leaf fleckings, similar to those of fire, on specimens stored in the Herbarium of the School of Botany, Cambridge, dated 1762 and 1847.

IN VITRO EXPERIMENTS WITH B. tulipae

Several tulip fire investigations have included *in vitro* experiments with *B. tulipae* done to corroborate field observations; Hopkins (1921), in giving a short account of growth in culture, noted that drying conditions favoured conidial production and Newton and Hastings (1931) tested a range of media to induce sporulation. Beaumont *et al* (1936) relied upon naturally produced conidia and sclerotia for their experiments but *en passant* observed that cultures exposed to bright daylight sporulated profusely, sclerotia being formed on similar cultures kept in darkness. Valaskova (1963 *a*, 1963 *b*) made extensive studies on *B. tulipae* and found that the illumination and nutrition of cultures were important factors in the formation of spores and sclerotia whereas temperature affected only mycelial growth rates.

To improve our understanding of the host-parasite complex and to provide standard inocula for experiments, some aspects of the biology of *B. tulipae* were studied *in vitro*. These *in vitro* experiments were directed primarily towards obtaining information to unravel the etiology of the disease.

A. Mycelial growth and sporulation.

(a) Differences between isolates and effects of temperature on growth. Most *Botrytis* species, with the notable exception of *B. cinerea*, have restricted host ranges but mixtures, often including *B. cinerea*, do occur and if undetected can cause misleading results which might be attributed to natural variation. To define the range of variation within *B. tulipae* growth rates of twelve isolates, obtained from a range of localities, were compared

at different temperatures. Three replicate petri dishes at each temperature, each containing 12 ml malt agar, were inoculated with 5 mm mycelial discs of each isolate and incubated at each of seven temperatures ranging from 2.5 to 28°C. Growth was measured daily along two diameters placed at right angles.

After an initial lag of c. 2 - 3 days daily growth became linear, the mean values for growth between days 3 - 4 (Table 1 & Fig. 1) show:

(1) that B. tulipae grew over a wide temperature range (c. 2.5 - 28°C, optimum 20°C), including temperatures commonly occurring in soils during winter and

(2) that considerable differences in growth rate exist between isolates.

To test the 'within-strain' variation, the growth of 20 cultures derived from different single mature conidia was assessed. Conidia from cultures 6 days old were tapped on to agar and 20 small agar blocks each containing one conidium (numbers of conidia/block were checked using a dissecting microscope) were placed centrally on petri dishes containing 12 ml malt agar before being incubated at 20°C in darkness. Two diameters of each culture were measured daily for 5 days, there being 20 different cultures of isolates GCRI 6, 17 and 29. Growth of all three isolates was conspicuously less during the first two days than at later stages (Table 2).

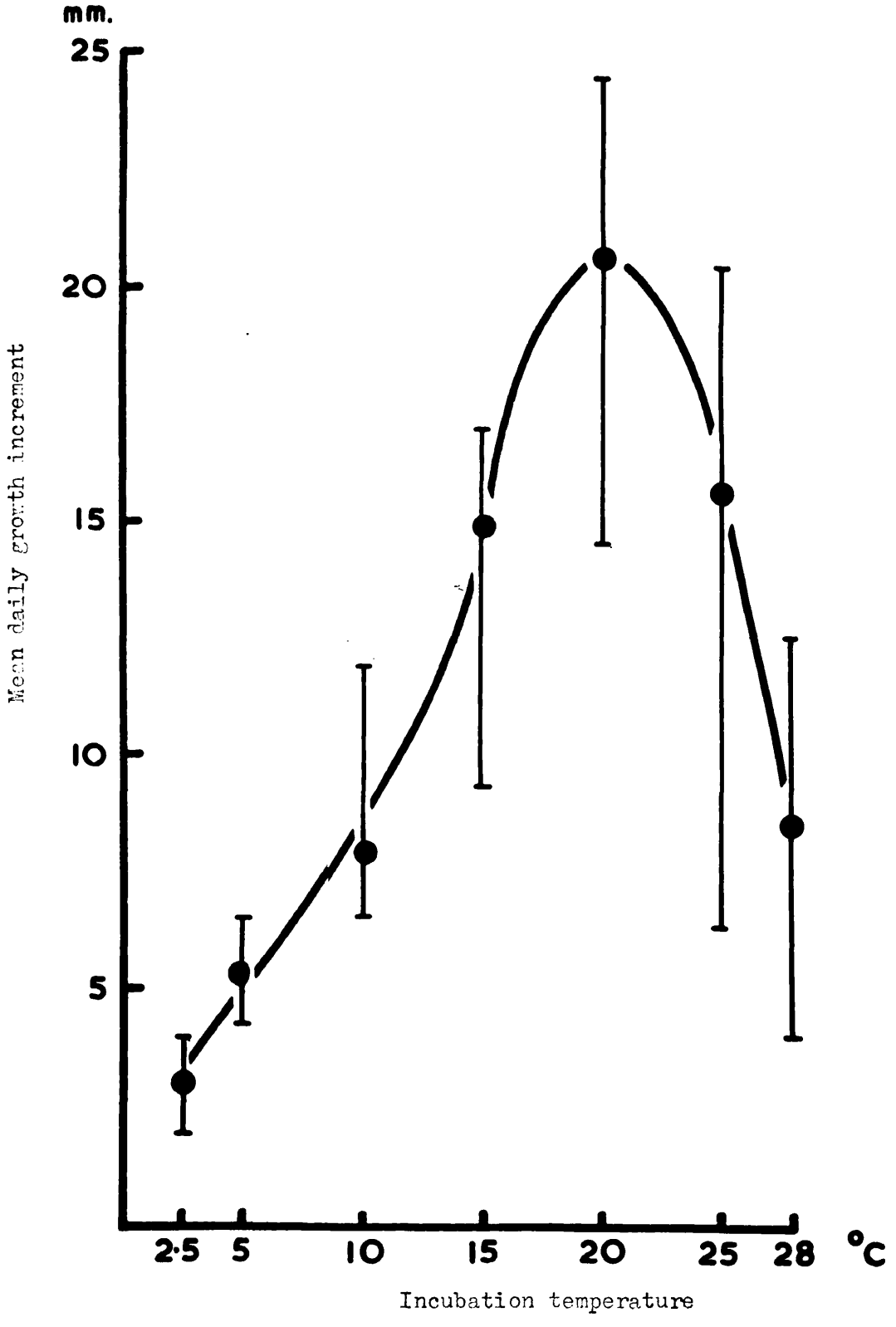
To eliminate statistical errors occurring when the standard errors (S.E.) of untransformed means are not independent, growth comparisons were made after transforming the daily diameters (x) to log (100 + x). The 20 single conidium cultures of 3 different isolates measured daily for 5 days produced 3 sets of 100 paired

Table 1

THE EFFECT OF DIFFERENT TEMPERATURES ON THE MYCELIAL GROWTH OF 12 DIFFERENT ISOLATES OF B. tulipae. DATA ARE MEANS OF THREE SETS OF PAIRED MEASUREMENTS OF DAILY GROWTH INCREMENTS (mm).

Source of isolate	Incubation temperature °C						
	2.5	5.0	10.0	15.0	20.0	25.0	28.0
United Kingdom							
Cambs. GCRI 6	4.0	5.6	7.7	15.7	22.7	18.5	7.5
Cornwall (a)	3.5	6.4	7.6	17.0	23.4	19.0	8.5
Cornwall (b)	3.0	6.1	7.2	16.0	19.4	19.8	6.4
Kent GCRI 17	2.5	5.9	10.0	15.3	24.0	15.7	4.0
Lincs.	4.0	5.5	7.0	15.8	19.8	8.7	6.6
Sussex	2.8	4.6	8.5	17.1	24.3	6.3	9.2
Yorks.	0.0	5.8	6.9	19.3	14.5	11.3	7.6
Guernsey	0.0	4.6	11.8	15.8	21.0	20.4	9.1
Czechoslovakia	4.0	4.6	7.9	15.0	21.3	18.8	14.7
Egypt GCRI 29	3.0	4.3	7.2	15.3	17.7	16.3	6.1
Netherlands (a)	3.0	4.3	6.6	11.7	18.8	16.8	12.5
Netherlands (b)	2.0	4.6	6.8	13.7	20.5	15.9	9.8
Mean values	3.0	5.2	7.9	14.8	20.6	15.6	8.5

Fig. 1. Mean daily growth increments of 12 isolates of B. tulipae at different incubation temperatures



observations for regression analysis. These were analysed by computer, the following regression equations being attained after de-transformation:

GCRI 6 (Cambs.) $y = 4.5 + 0.124098\ x$

GCRI 17 (Kent) $y = 4.5 + 0.149617\ x$

GCRI 29 (Egypt) $y = 4.5 + 0.119811\ x$

The regression coefficients 0.124098 and 0.119811 of GCRI 6 and 29 respectively are similar, as judged by a 't' test, but that for GCRI 17 is significantly different, growing more rapidly than the others (Fig. 2).

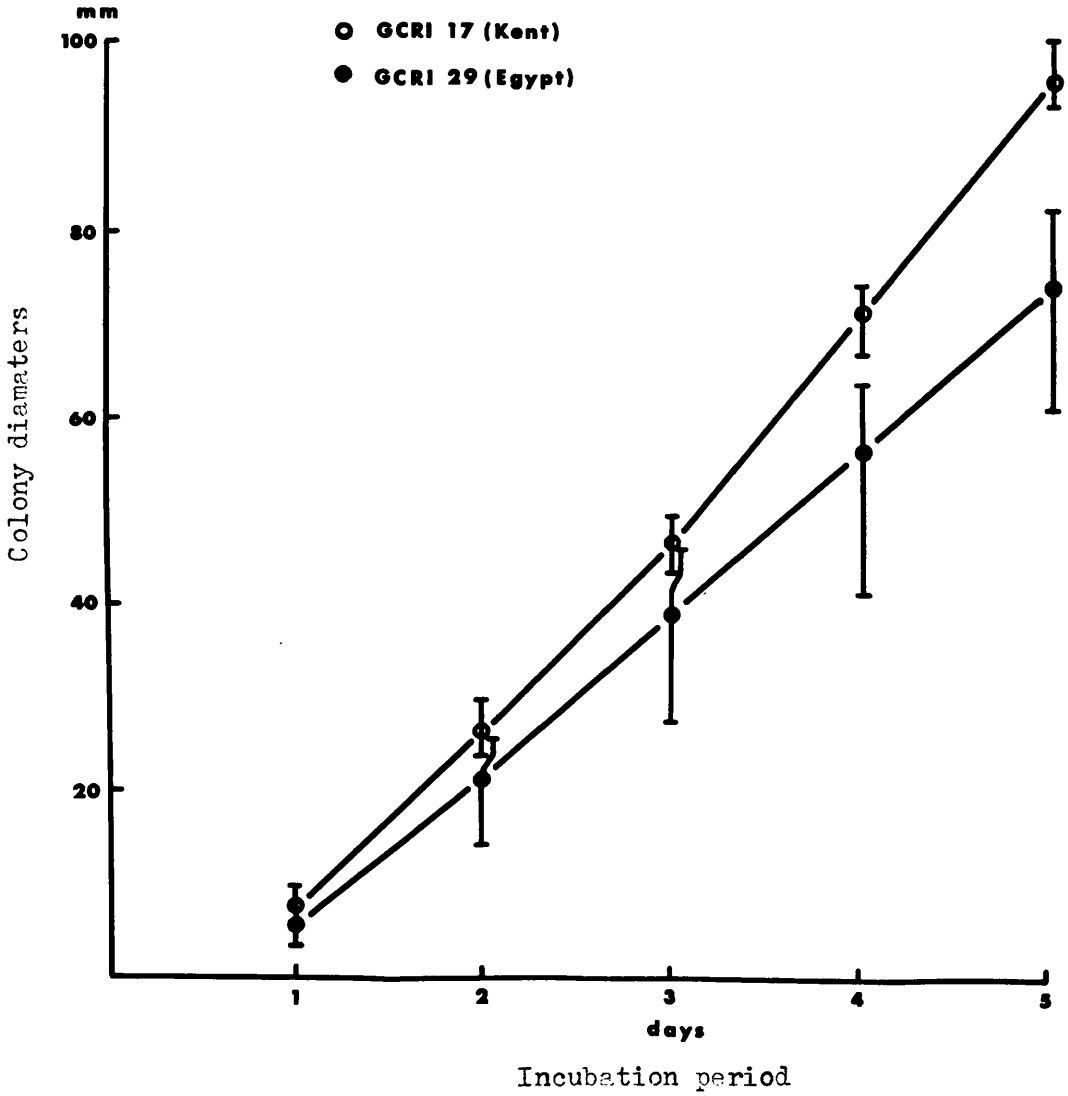
Table 2

GROWTH DIFFERENCES BETWEEN SINGLE SPORE CULTURES OF 3 DIFFERENT *B. tulipae* ISOLATES, WHEN GROWN ON MALT AGAR AND INCUBATED IN DARKNESS AT 20°C

	Accession No. of *B. tulipae* cultures		
	6	17	29
Source	Cambs.	Kent	Egypt
	Mean daily diameters (mm)		
Day 1	6.5	7.2	3.2
2	20.8	27.4	11.7
3	39.0	46.4	27.1
4	56.1	70.9	42.7
5	74.4	95.2 *	64.4

* Extrapolated value

Fig. 2. Changing diameters of 20 replicate single conidial isolates of 2 cultures of B. tulipae when grown on malt agar at 20°C in darkness.



(b) Effects of nutrition and light on B. tulipae. Valaskova (1963 a, 1963 b) examined in detail nutritional requirements of B. tulipae. She noted that sporulation was increased by light in the blue waveband and that asparagine and glucose together were the most favourable energy sources. Combinations of small amounts of nitrogen and large amounts of carbohydrate favoured sclerotia formation; the converse favouring sporulation. She estimated conidial numbers on an arbitrary scale of 0, indicating the absence of conidia and 4, their profuse development, but in my experiments numbers of conidia were estimated by one of two methods:

(1) Washing: by washing the surface of B. tulipae cultures with 5 ml of a 0.02% Triton X-100 aqueous solution, shaking by hand, filtering the suspension through two layers of 15 denier nylon mesh and making up the filtrate to 25 ml; conidia being counted in aliquots mounted in a Neubauer haemocytometer.

(2) Tapping: conidia were tapped from cultures before being suspended in 25 ml water, the suspensions being shaken by hand before being filtered through cotton wool; numbers of conidia in the filtrate being counted in several droplets of known volume.

Comparing growth and conidial production by B. tulipae grown on three sources of nitrogen in light and darkness confirmed Valaskova's observations. Three batches of modified Czapek-Dox agar were made using 1.56 g asparagine, 2.00 g sodium nitrate or 1.56 g ammonium sulphate/l. to give equal amounts of nitrogen, i.e. 0.2% N. Ten petri dishes of each N source were inoculated with 5 mm discs of mycelium; five being kept in darkness, both

groups were incubated at 20°C (Table 3).

Table 3

EFFECT OF LIGHT ON NUMBERS OF B. tulipae CONIDIA FORMED ON MEDIA CONTAINING DIFFERENT NITROGEN SOURCES

Nitrogen source in agar media	Incubation continuous in	
	dark	light (110-120 lumens/ft^2)
	Number of conidia/cm^2 of culture	
Asparagine	0.64	1093.0
Sodium nitrate	0.22	122.0
Ammonium sulphate	0.14	139.0
Mean	0.33	451.4

More conidia were produced on media with asparagine than with ammonium or nitrate N (x 3 in dark, x 8 in light) but the effect of nitrogen source was relatively small compared with that of light. The mean effect of light was to increase sporulation by a factor of more than 1000, from 0.64 to 1093 conidia/cm^2, irrespective of nitrogen source.

Although large numbers of conidia were produced on illuminated cultures growing on Czapek-Dox medium with asparagine the ease of preparation of malt agar (15g malt extract, 25g agar/l.) which also contains organic nitrogen, commended itself for further experimental work.

Freshly isolated cultures of B. tulipae sporulated well on illuminated malt agar but numbers of conidia gradually decreased with continual sub-culturing. The ability to spore was maintained by occasionally sub-culturing on Czapek-Dox agar.

Bjornsson (1956) examined in detail the effect of light on B. gladiolorum showing that when periods of light exceeded 7 hr the intensity of sporulation increased. He found that this response was associated with near-UV wavelengths, a result in agreement with those of Leach (1962), who found that B. cinerea cultures continuously irradiated at 76 microwatts/cm^2 with 3100 - 4000 Å light for 3 - 10 days sporulated profusely. Leach also showed that when the near-UV wavelengths in daylight fluorescent tubes were filtered out, sporulation was reduced or ceased.

Although neither Bjornsson's nor Leach's experiments were repeated exactly with B. tulipae the effects of lengthening daylength (Table 4) and increasing light intensity (Table 5) were examined. Petri dishes containing malt agar were inoculated centrally with a sclerotium, 5 dishes then being exposed to daylengths of 8, 16 and 24 hr, the light source being two 4 ft 80 W Mazda warm white fluorescent tubes giving a light intensity at culture level of 110 - 120 lumens/ft^2.

After 10 days, numbers of conidia/cm^2 of culture were determined by the tapping method described on p 10. Increasing daylength increased conidial numbers (Table 4) but there was no great effect of different light intensities (Table 5).

(c) Effects of culture age on sporulation. The extremely variable numbers of conidia produced on replicate cultures are a serious disadvantage in the preparation of standard inocula, where, to achieve uniformity, it is preferable to avoid mixing conidia from different cultures. This could only be achieved if cultures were used when conidial production was maximal.

Table 4

NUMBERS OF B. tulipae CONIDIA FORMED ON PETRI DISHES OF MALT AGAR EXPOSED FOR 10 DAYS TO DIFFERING DAYLENGTHS.
(EACH PLATE INOCULATED WITH A SCLEROTIUM)

Daylength *	Numbers of conidia/cm^2 of culture
8 hr	7.7×10^2
16 hr	11.6×10^2
24 hr	13.6×10^2

* During the appropriate period the light intensity was 110 - 120 lumens/ft^2.

Table 5

EFFECT OF TWO LIGHT INTENSITIES ON NUMBERS OF B. tulipae CONIDIA FORMED ON MALT AGAR CULTURES INCUBATED FOR 10 DAYS AT 20°C.

Light intensities	Numbers of conidia/cm^2 of culture
50 - 60 lumens/ft^2	4.7×10^3
110 - 120 lumens/ft^2	6.1×10^3

The increasing numbers of conidia formed with time was followed by inoculating petri dishes containing 12 ml malt agar with 5 mm discs of B. tulipae mycelium and incubating at 14.4°C with an 18 hr daylength of 110 - 120 lumens/ft^2 produced by warm white fluorescent tubes. At daily intervals 3 cultures were removed and conidial numbers estimated using the 'washing' method. Conidial production commenced when the cultures were 6 days old, reaching a maximum after 12 days (Table 6). The age of conidia from 12 day old cultures would vary too much for use as inoculum. A technique was required which would provide a large number of conidia/dish approximately 1 day old.

Table 6

DAILY INCREASES IN NUMBERS OF B. tulipae CONIDIA FORMED ON MALT AGAR CULTURES GROWN IN CONTINUOUS FLUORESCENT LIGHT AT 14.4°C.

Age of culture	Relative numbers of conidia *
5 days	0.0
6	1.2
7	4.6
8	27.7
9	21.1
10	-
11	74.4
12	92.3

* Suspensions prepared in water and haemocytometer counts made.

(d) Comparison of conidial production methods. Unfortunately the use of the 'washing' method with 12 day old cultures has several disadvantages, viz.:

(1) Although suspensions of conidia from washed cultures contain many mature conidia, some inevitably will be immature and possibly less infective so confusing results from leaf inoculation experiments.

(2) Germination commences with the wetting of the conidia and experiments have shown that germ tubes are formed after c. 90 min leaving little time for filtering and adjusting inoculum concentration.

(3) The addition of a surface active agent to decrease conidial clumping and increase counting accuracy causes, concomitantly, droplet spread on leaf surfaces.

To increase the production per petri dish of conidia of the same age, three inoculation procedures were tested:

(1) Multipoint inoculation by tapping dry conidia from a 6 day old culture - experience with a micromanipulator had shown that only mature conidia are easily detached.

(2) Single point inoculation

a. with conidia and,

b. with sclerotia.

The treatments were replicated thrice, petri dishes being incubated for 6 days under continuous warm white fluorescent light (110 - 120 lumens/ft^2) at 14.4°C.

The production of mature conidia was assessed using the 'tapping' method described on p 10. Cultures derived from sclerotia produced few conidia, probably because of time taken to germinate, but numbers from multipoint inoculated dishes were double those

Table 7

EFFECT OF DIFFERENT TYPES OF *B. tulipae* INOCULA ON CONIDIAL NUMBERS FORMED ON MALT AGAR WHICH WAS SUBSEQUENTLY INCUBATED FOR 6 DAYS IN CONTINUOUS FLUORESCENT LIGHT AT 110-120 LUMENS /FT^2

Types of inocula	Replicate	Numbers of conidia/ cm^2 of culture	Mean
1. Multipoint :	1	12.5	
Numerous scattered	2	14.0	14.3 ± 1.42
dry conidia	3	16.4	
2. Single point :	1	3.6	
(a) single conidium	2	9.3	6.1 ± 1.46
	3	5.5	
(b) single sclerotium	1	0.9	
	2	0.8	0.6 ± 0.18
	3	0.4	

inoculated with only one conidium (Table 7). This method of multipoint inoculation was developed so that numbers of conidia/ plate were increased, and the ability to produce large numbers of similarly aged conidia made possible leaf inoculation experiments with less variable results than would have otherwise been the case.

(e) Preparation of inoculum. Speed and accuracy are required when preparing inocula and it was necessary to know if inocula of different concentrations could be mathematically predicted when diluting. Dense suspensions of conidia were prepared in 20 ml water and counts made of numbers in five 0.002 ml drops. Conidial concentrations were checked in a dilution series with steps of x $\frac{1}{2}$ and it was found that concentrations were usually greater than expected (Table 8), a feature previously recorded by Meiklejohn (1957). As a result the desired concentrations of inocula were always prepared from actual counts.

B. Conidial germination.

Gottlieb (1950) listed eleven factors likely to influence germination, which were dominated by the availability of water at a suitable temperature. Spore germination has been studied in considerable detail in fungicide assay work where assessments were usually based upon counts made 24 hr after preparing spore suspensions, fungicidal efficacy being judged on an all-or-none basis, spores either dying or remaining dormant (American Phytopath. Soc. 1947). In the etiology of a disease such as tulip fire, however, it is important to characterise rates of germination, an aspect that was emphasised when testing the influence of different factors.

Table 8

NUMBERS OF *B. tulipae* CONIDIA COUNTED IN SERIAL WATER DILUTIONS COMPARED WITH EXPECTED VALUES

Dilution	Conidia/ml x 10^3	
	Mean no. counted *	Expected number
Initial suspension (N)	35.9 ± 2.58	36.00
0.5 N	26.4 ± 2.08	18.00
0.25 N	14.0 ± 1.09	9.00
0.125 N	10.9 ± 0.49	4.50
0.0625 N	7.4 ± 0.54	2.25
0.0317 N	6.0 ± 0.65	1.12
0.0158 N	1.4 ± 0.19	0.56
0.0079 N	1.2 ± 0.30	0.28
0.0035 N	0.8 ± 0.20	0.14

* Mean numbers of conidia in 5 x 0.002 ml drops.

What happens when conidia of B. tulipae start to germinate? Before experiments were made to test factors influencing germination, the process of germination itself was examined.

By combining the hydrolysing and staining methods of Knox-Daviss and Dickson (1960) and Hrushowetz (1956), it became relatively easy to count nuclei - the chromosomes being darkened. Using Roane's method (1952), wet conidia were brushed on to previously autoclaved pieces of cellophane and allowed to germinate. After predeterminate periods of incubation, conidia adhering to the cellophane squares were fixed overnight in a 3:1 mixture of absolute alcohol and glacial acetic acid. After fixing, conidia, still attached to cellophane, were rinsed in water then immersed in cold N HCl for 5 min, hydrolysed in N HCl at 60°C for 7 min, washed thoroughly in distilled water, rinsed in phosphate buffer (pH 6.9), and placed in Giemsa stain for 2 hr. The phosphate buffer was prepared by adding M/5 NaOH to 50 ml M/5 KH_2PO_4 to bring pH to 6.9, then the volume was increased to 200 ml with distilled water. A solution of Giemsa stain was prepared by adding 25 ml glycerol to 0.38 gm of the stain already dissolved in 25 ml methanol. The mixture was warmed in a plugged flask for one hour before cooling and filtration. Five ml phosphate buffer with 10 drops of stain were poured on each cellophane square, the excess stain being removed after 2 hr by rinsing with water.

Germination, recorded when germ tubes were at least as long as the conidial width, started 90 min after preparing water suspensions at ambient temperature and before this time numbers of nuclei increased from 3.0 to 6.2 (Table 9), the most rapid increase being in the first 30 minutes. Germ tubes with only 2 nuclei appeared between 90 and 120 min, numbers of nuclei

Table 9

CHANGING NUMBERS OF NUCLEI IN CONIDIA OF B. tulipae GERMINATING IN STERILE DISTILLED WATER

	Period of incubation		Mean no. nuclei in conidia
A.	Pre-germination (a)*		
	0		3.0 ± 0.25
	30 min		5.4 ± 0.29
	60 min		5.3 ± 0.65
	90 min		6.2 ± 0.20
B.	Post-germination		
	2 hr		2.4 ± 0.14
	4 hr		3.2 ± 0.18
	6 hr		5.4 ± 0.17
	8 hr	1) (b)*	7.8 ± 0.26
		2)	3.9 ± 0.31

(a)* Germination was judged to have taken place when the germ tube was at least twice as long as the width of the conidium.

(b)* Cell wall formed in germ tube between 6 and 8 hr after suspensions were prepared. 1) and 2) Mean nuclei number in terminal and penultimate cells respectively.

subsequently increasing. Cell walls first appeared 8 hr after germ tube emergence when there were 7.77 and 3.91 nuclei in terminal and penultimate cells respectively. The presence of 3 - 4 nuclei in dormant conidia and non-apical cells suggests that there is great potential for variation; variation that has been shown to exist in growth rates. Hastie (1964) has shown,using Verticillium albo-atrum Reinke and Berth., that the single nuclei found in conidia are the products of division in the phialides and, by using nutritional genes as markers, that the frequency of mitotic recombination was considerable.

(a) Effects of water vapour. Gottlieb (1950) considered that water was an important germination trigger but the need for liquid or vapour phase differed with different species.

Dry conidia of B. tulipae were tapped from 6 day old cultures on to glass cover slips, placed in chambers (Jarvis 1960) with atmospheric humidities controlled at 80, 90 or 100% R.H. (Carson 1931). Whereas 76% of the conidia germinated within 24 hr at 100% R.H., fewer conidia germinated at lower humidities (Table 10).

Table 10

EFFECTS OF DIFFERING ATMOSPHERIC HUMIDITIES ON THE GERMINATION OF B. tulipae CONIDIA DURING 24 HOURS' INCUBATION AT 20°C

Atmospheric humidity		% germination*
Relative Humidity %	Saturation Vapour Deficit (mb)	
100	0.00	76
90	2.26	17
80	4.55	0

* Coverslips seeded with dry conidia.

(b) Effects of different periods of storage. Last & Hamley (1956) using B. fabae, showed that conidia taken from old cultures, although able to germinate, were less able to infect broad bean leaves than conidia from young cultures. My method of assessing germination was to tap dry conidia on to the petri dish lid, wash and shake in distilled water before filtering and adjusting to the required concentration in 50 ml containing 0.05 g glucose as a stimulant. Five replicate 0.002 ml drops of conidial suspension were placed on clean glass microscope slides and incubated in damp chambers (petri dishes lined with damp filter paper) in darkness. All conidia in each drop were counted. Because these drops evaporate quickly in the laboratory atmosphere where the assessments were made, drops were only counted once, different drops being used for counts made after different periods of incubation.

Dry conidia of B. tulipae from illuminated malt agar cultures were tapped on to petri dish lids and stored for 7, 14 and 21 days after forming chambers with the two components of petri dishes. After appropriate storage periods, conidia were suspended in water and counts of germinated and ungerminated conidia made after 8 hr incubation. These indicated that the ability to germinate greatly decreased after two weeks storage (Table 11).

(c) Effects of temperature. Several problems were overcome in determining temperature effects on conidial germination. The use of small drops containing few conidia was favoured because these more nearly simulated natural infection drops on leaves. Because of

(1) rapid evaporation of drops when counting,

(2) temperature rise of cooled drops when being counted, and

(3) germination of conidia at optimal temperatures,

Table 11

EFFECTS OF DIFFERENT PERIODS OF STORING DRY CONIDIA OF *B. tulipae* ON THEIR SUBSEQUENT % GERMINATION

Periods of storage*	Detailed results from one experiment						Mean of 4 exps.
	Individual replicates					Mean of 5 replicates	
	1	2	3	4	5		
7 days	47	20	24	16	32	28	37
14 days	50	90	54	62	47	61	57
21 days	0	0	0	0	0	0	0.5

* Conidia suspended in water at the end of their storage periods and % germination determined after 8 hr incubation.

Table 12

EFFECTS OF DIFFERENT TEMPERATURES ON THE RATE AT WHICH *B. tulipae* CONIDIA GERMINATED (CALCULATED FROM PROBITS).

Period of incubation	Temperature °C				
	5	10	15	20	25
2 hr	0.0	0.0	8.9	15.9	19.8
4 hr	0.0	0.0	42.1	52.8	74.3
6 hr	0.0	15.9	67.4	88.9	94.0
10 hr	6.7	50.0	89.8	99.3	-
14 hr	22.7	78.8	96.4	-	-
18 hr	44.0	89.4	-	-	-
22 hr	61.8	95.6	-	-	-
26 hr	75.2	-	-	-	-

the data on germination have been compiled from several experiments covering the range of temperatures. By transforming values of % germination and the time scale to logarithms, there was a linear relation from which intermediate values were calculated (Table 12). At each temperature, germ tube appearance was preceeded by a lag phase shortening from c. 8 hr at 5°C to less than 4 hr in the range 15 - 25°C. Germination proceeded rapidly in the 15 - 25°C range, being largely complete by 6 hr but at lower temperatures germination was markedly slower - the time taken for 10, 50 and 75% germination at different temperatures being plotted in Fig. 3. The set of curves emphasises the long periods necessary for germination at the lower temperatures (i.e. below 15°C), possibly suggesting why fire develops slowly until late spring when atmospheric temperatures appreciably increase.

(d) Effects of suspensions of different concentrations. Four conidial suspensions containing similar amounts of glucose were made with 1 to 27 x 10^3 conidia/ml (Table 13). The analysis of variance assumes normal distribution of data but this does not occur with percentages and for the purposes of statistical analysis these germination values were transformed to angles. As concentrations decreased the proportion of conidia germinating, during 7 hr incubation, increased. Why should this be?

To test the suggestion that germinating conidia exude inhibiting substances, a conidial suspension with 45 x 10^3 conidia/ml was prepared. After germination conidia and germ tube fragments were removed by filtration, the filtrate being used in the preparation of a new suspension and some of the new conidia being suspended in distilled water for comparison.

Fig. 3. Time taken by 10, 50 and 75% of B. tulipae conidia to germinate in water drops at different temperatures.

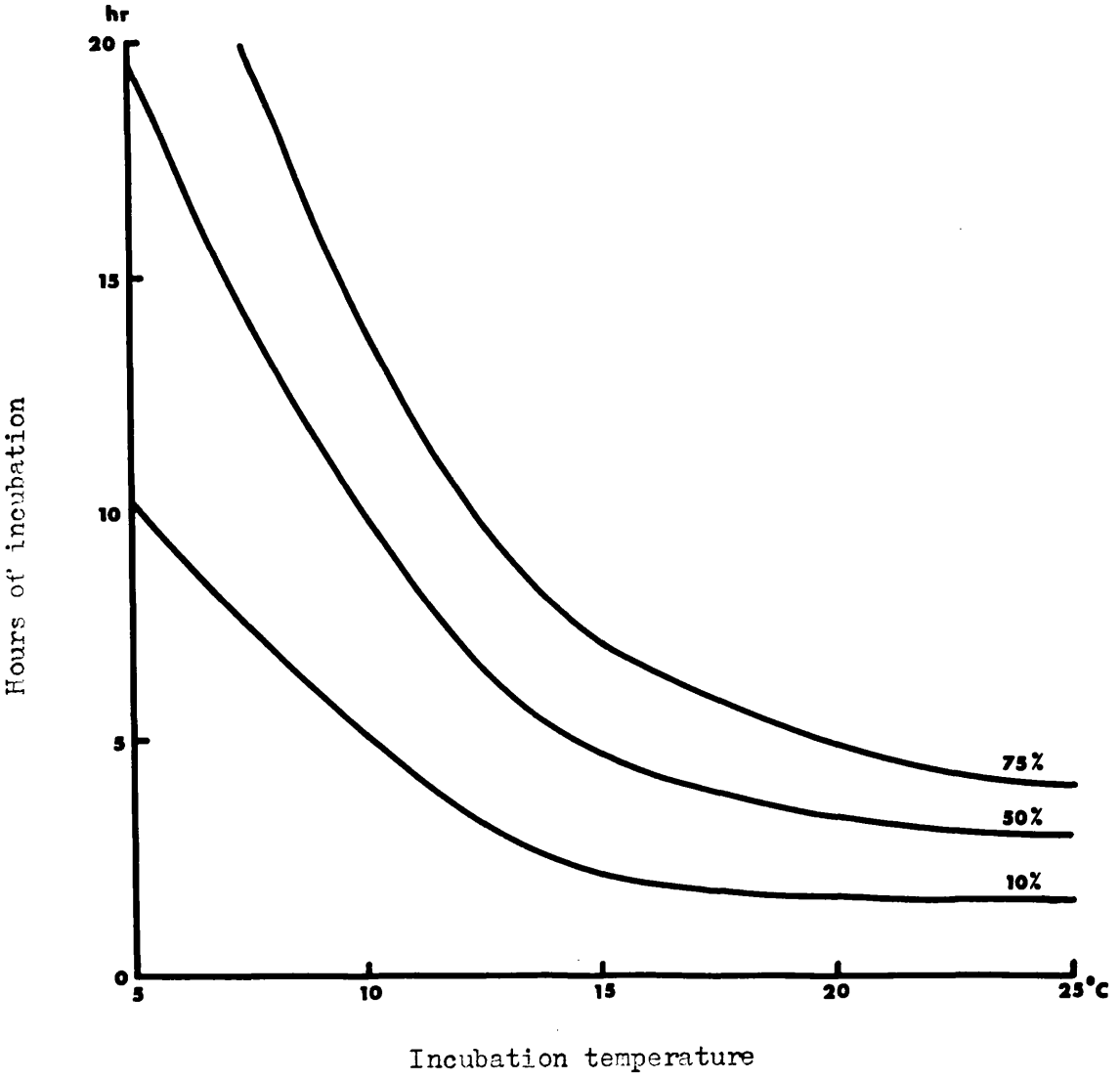


Table 13

GERMINATION OF B. tulipae CONIDIA IN SUSPENSIONS OF DIFFERENT CONCENTRATIONS.

Mean no. of conidia/ 0.002 ml drop of suspension	Concentrations of conidia/ ml	Mean % germination
5.8 ± 0.58	1.4 x 10	96.1 (78.61)*
21.9 ± 1.70	5.5 x 10	90.9 (72.44)
50.5 ± 1.72	1.3 x 10	88.7 (70.36)
109.0 ± 4.62	2.7 x 10	82.9 (65.57)

L.s.d. (p = 0.05)(6.22)

L.s.d. (p = 0.01)(8.37)

* For analysis, data were transformed to angles which are underlined.

Table 14

EFFECTS OF CONIDIAL EXUDATES ON THE GERMINATION OF TWO DIFFERENT CONCENTRATIONS OF B.tulipae CONIDIA SUSPENDED IN EITHER WATER OR THE FILTRATE AFTER ELIMINATING CONIDIA WHICH HAD GERMINATED IN THE PREVIOUS 24 HR.

Suspending medium	Concentrated fresh suspension		Dilute fresh suspension	
	Conidia/ 0.002 ml drop	% germination	Conidia/ 0.002 ml drop	% germination
Conidial exudate	88.4 ± 3.05	5.6	15.3 ± 2.00	19.8
Distilled water	101.4 ± 3.71	89.9	14.3 ± 1.99	71.1

Fewer conidia germinated in the filtrate from an earlier batch of germinating conidia than in water, suggesting conidia exude a water soluble inhibitor (Table 14).

(e) Effects of tulip leaf. Although germination is favoured by humid conditions it is surprising that the axillary regions of tulip leaves, where rain and dew collect, are not more severely attacked than other sections of leaf. Does the tulip influence conidial germination? Rain water, remaining overnight in the axils of young undamaged tulip leaves without abundant microfloras, was collected and used for preparing conidial suspensions. Germination counts made after 6.5 and 24 hr incubation show that conidia germinated less rapidly in rain water collected from tulip leaves than in distilled water, the effect being relatively greater after 6.5 hr than at the end of 24 hr (Table 15).

Table 15

EFFECTS OF POSSIBLE LEACHATES FROM TULIP LEAVES ON THE GERMINATION OF *B. tulipae* CONIDIA

Suspending medium	No. of conidia/ 0.002 ml drop	Period of incubation	
		6.5 hr	24 hr
		% germination	
Leachate*	96.5 ± 3.13	8.0	77.0
Distilled water	85.4 ± 1.63	26.1	91.8

* Rain water collected from tulip leaf axils after standing overnight.

Plates 1 & 2.

Plate 1.

Aggressive and
non-aggressive lesions
on leaf.

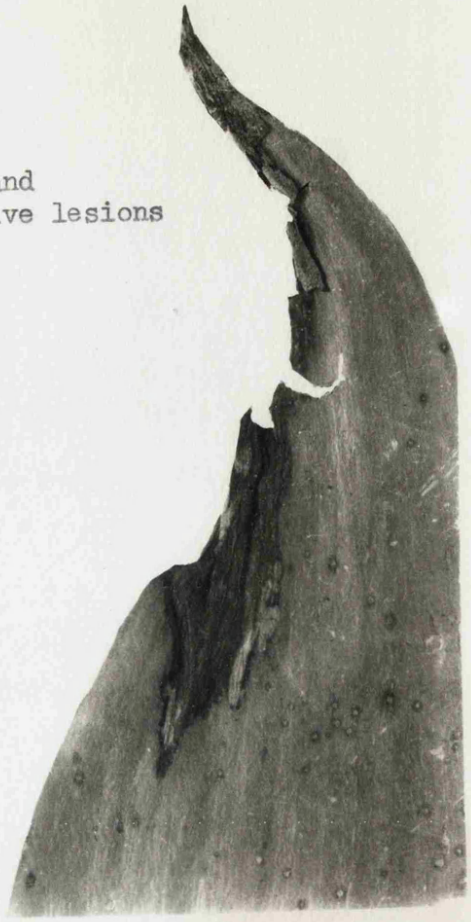


Plate 2. Aggressive and non-aggressive lesions on flowers.

ABOVE-GROUND ETIOLOGY OF TULIP FIRE

A. Tulip and pathogen interrelations.

(a) Types of lesion occurring on leaves and flowers. Two types of spotting occur on leaves and flowers of field grown crops:

(1) spots remaining small, yellowish-brown with peripheral water soaking (Plate 1 & 2) and

(2) spots enlarging, their centres becoming depressed and whitish-grey, forming a substrate on which B. tulipae, in some weather conditions, sporulates profusely (Plate 1 & 2).

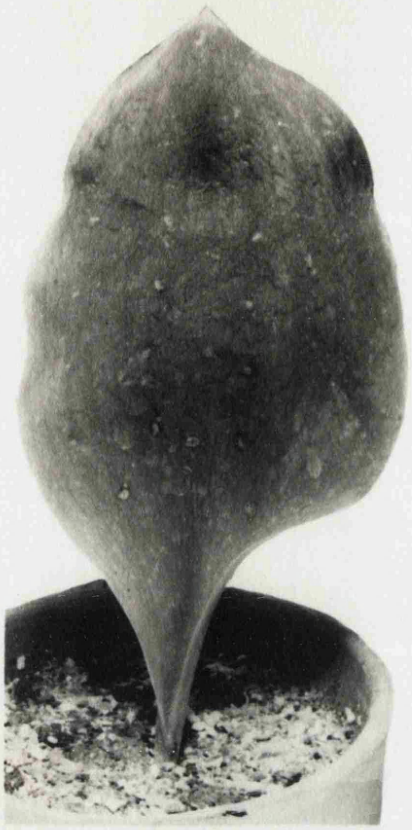
Hopkins (1921) did not separate these two types of lesion considering that all spots of type (1) were capable of subsequent enlargement, they therefore being successive stages in the development of one and the same infection. Beaumont et al (1936) labelled Hopkin's symptom (1) as 'spot' and (2) as 'fire'. Wilson (1937), in his studies on chocolate spot of beans, caused by B. fabae Sard., noted a similar range of lesions, calling the discrete spots 'non-aggressive' and labelling the enlarging lesions 'aggressive', having shown that the latter developed as a result of inoculating with more concentrated spore suspensions.

Among tulip shoots emerging in spring are so-called 'primaries', which are shoots already infected with B. tulipae and on which the pathogen characteristically sporulates profusely. The spread of B. tulipae in the field is often visible soon after the appearance of primaries with numbers of non-aggressive lesions decreasing with the increasing distance from these foci of infection. Because of the difficulties of detection it is rare for fieldmen to eliminate (rogue) primaries until zones of infected plants

Plates 3 & 4.

Plate 3.

Typical maiden leaf used for inoculation experiments.



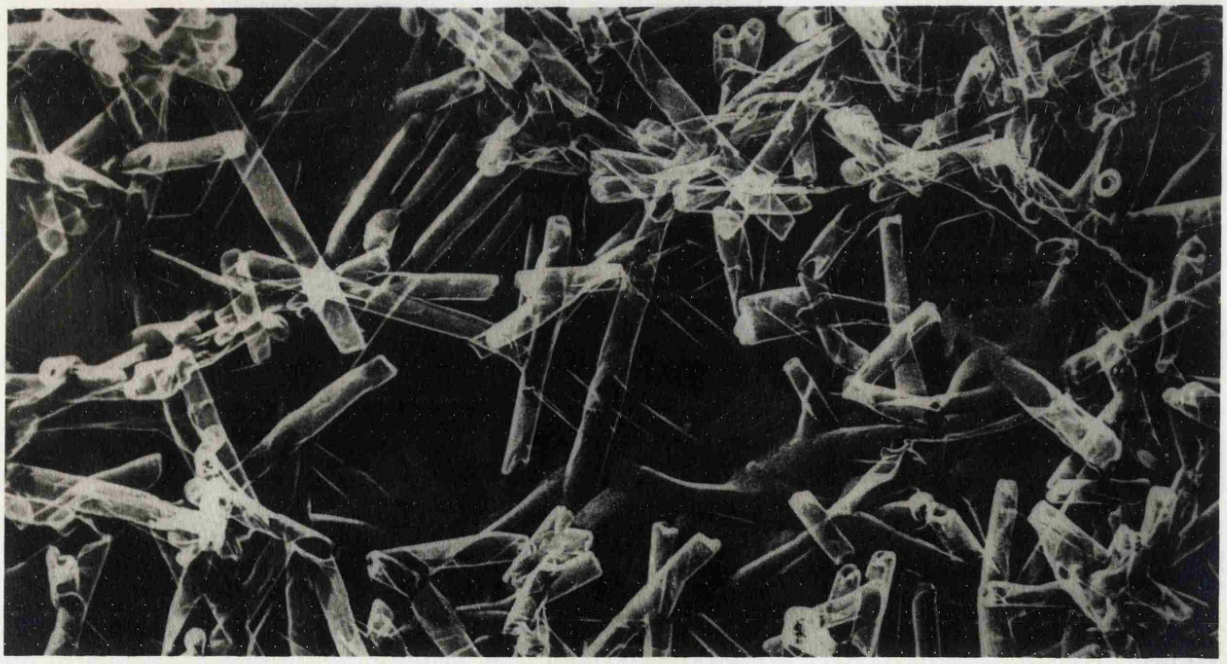


Plate 4. Abaxial surface of tulip leaf showing randomly scattered cylinders of plant wax. (x 18000).

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measure 3 - 6 ft in diameter: when aggressive lesions have usually appeared on plants nearest to the sources of inocula. It is from these aggressive lesions that new generations of B. tulipae conidia are dispersed by wind and water splash. In epidemics multi-infections cause extensive leaf scorch with both types of lesion.

(b) Factors affecting formation of non- aggressive lesions.

Lesion formation is influenced by:

(1) external factors i.e., presence or absence of epidermal wax, other leaf inhabiting microbes, duration of water films, and

(2) internal factors i.e., ageing, nutrition and water stress.

Uniform batches of leaf material for inoculation experiments were obtained from small maiden bulbs which develop single large leaves (Plate 3). These bulbs were grown in J.I.1. potting compost in 3 in clay pots arranged closely together to ensure similar environment and microbial colonisation. The plants were well watered the day before use to avoid possible water stresses. Unless otherwise stated the leaves were gently wiped with damp cotton wool to remove superficial waxes and placed in a saturated atmosphere immediately after inoculation for 48 hr.

Conidial inocula were prepared as already described in the section on conidial germination, measured drops of conidial suspension being applied using an 'Agla' syringe fitted with a micrometer and a 214 L needle, replicate drops having first been counted on glass slides.

(1) Leaf surface waxes. When unfurling, tulip leaves repel water - an effect that photomicrographs have shown to be associated with small cylinders of randomly scattered superficial

plant wax (Plate 4). Water droplets tend to bead, rolling off leaf laminae and into cups formed where leaf bases and stems meet. In the field, 'waxiness' is easily removed when plant tissues rub against each other. Circumstantial evidence suggests that populations of yeasts, capable of eroding cutin, colonise leaf surfaces and together these biological and physical influences may act to appreciably increase the ease of foliar wetting. The inhibitory association of waxiness is shown in Table 16. Inoculated plants (15 separate drops of inocula/leaf) were kept in a saturated atmosphere for 48 hr at which time lesions were not visible, but after a further 24 hr in the drier atmospheres of the laboratory (c. 80% R.H.) lesions developed, more on leaves without wax than on those with wax, a result paralleled by the effects recorded in germination experiments (p 24). Where wax was removed, conidia in larger drops were more infective than those in smaller drops, possibly explained by the self inhibition of germinating conidia shown on p 26. In one of the other replicate experiments the effect of waxiness and drop size suggest lesion development increases with time, irrespective of drop size, reaching maxima in c. 6 days, (Table 17).

(2) Post-inoculation atmospheric humidity. Lesions developed when leaves were kept in saturated atmospheres for 24 hr immediately after inoculation but none occurred when this period was decreased to 6 hr (Table 18). To narrow the limits of the essential humid period of high humidity 8 plants were inoculated each with 30 drops of inoculum, one pair being kept in the drier laboratory atmosphere and the other 3 pairs placed in a saturated atmosphere immediately after inoculation. Pairs of plants were removed from this high humidity to the laboratory atmosphere after

Table 16

ASSOCIATION OF LEAF WAXES AND SIZE OF INOCULUM DROP WITH NUMBERS OF LESIONS DEVELOPING ON LEAVES AFTER 3 DAYS

(15 drops/leaf containing c. 20 conidia/drop irrespective of size)

Drop size	Nos. non-aggressive lesions	
	Leaf with wax removed	Leaf with wax
Experiment 1*		
0.001 ml	5	1
0.002 ml	4	2
0.003 ml	13	5
TOTAL	22	8
% successful of 45 drops	49	18
Experiment 2		
% successful of 40 drops	50	12

* To minimise variation in all experiments leaves were divided into 2 longitudinal sections or 4 sectors, inoculation treatments being tested in each half or a 4 x 4 Latin square.

Table 17

EFFECTS OF LEAF WAXES AND SIZE OF INOCULUM DROP ON RATE OF LESION DEVELOPMENT

(10 drops/leaf containing c. 15 conidia/drop irrespective of size, there being 4 replicate leaves).

	Nos. days after inoculation					
	2	6	8	2	6	8
Drop size	leaf with wax removed			leaf with wax		
0.001 ml	5	5	5	1	3	3
0.002 ml	6	10	10	0	1	2
0.003 ml	4	7	7	0	3	3
0.004 ml	2	11	11	0	3	3
TOTAL (of 40)	17	33	33	1	10	11

Table 18

EFFECTS OF DIFFERING PERIODS OF POST-INOCULATION HUMIDITY ON NUMBERS OF LESIONS FORMED FROM DIFFERENT SIZED INOCULUM DROPS

(200 drops/leaf containing c. 12 Conidia/drop, arranged on 4 leaves)

	Duration in saturated atmosphere			
	6 hr		24 hr	
Drop size (ml)	0.001	0.002	0.001	0.002
	numbers of non-aggressive lesions			
Replicates	0	0	42	83
	0	0	16	37
TOTAL	0	0	58	120
% of 200 inoculations where lesions developed	0	0	29	60

18, 24 and 48 hr, lesions being counted at these intervals (Table 19). No lesions developed on leaves kept continuously in dry atmospheres, the conidia probably not germinating (vide p 21). On the other hand lesions developed at 60% of the inoculation sites when incubated for 18 hr at high humidities.

Table 19

NUMBERS OF NON-AGGRESSIVE LESIONS FORMED ON LEAVES KEPT IN SATURATED ATMOSPHERES FOR 4 DIFFERENT DURATIONS. (30 drops/leaf containing 30 conidia/0.002 ml drop, 2 plants/treatment.

	Period of saturated atmosphere (hr)			
	0	18	24	48
Time observations made after inoculation.	No. of lesions developing of a possible 60.			
18 hr	0	27	37	34
24 hr	0	27	42	52
48 hr	0	27	42	52

(3) Leaf age. In a later section it will be shown that B. tulipae remains viable within lesions for protracted periods. As disease incidence usually increases towards the end of cropping seasons it is possible that the activity of established infections might be influenced within leaves by effects of increasing age. To explore this possiblity identical experiments testing different concentrations of inocula were done in early April and again in mid-May when the batch of plants was one month older and with visible leaf tip scorch - an early sign of senescence. The data suggest that leaf senescence has little or no effect on the formation of non-aggressive or aggressive lesions (Table 20a & b).

Table 20 a

NUMBERS OF NON-AGGRESSIVE LESIONS, EXPRESSED AS PERCENTAGES FORMED BY DIFFERENT INOCULUM CONCENTRATIONS ON NORMAL AND SENESCING LEAVES

Period of incubation	Nos. conidia/drop			
	20	30	40	50
1. No leaf senescence (early April)				
2 days	64	-	-	80
2. Slight leaf senescence (mid May)				
1 day	0	33	7	4
2 days	47	84	44	51
3 days	49	87	51	60

Table 20 b

NUMBERS OF AGGRESSIVE LESIONS, EXPRESSED AS PERCENTAGES OF NON-AGGRESSIVE LESIONS, FORMED BY DIFFERENT INOCULUM CONCENTRATIONS ON NORMAL AND SENESCING LEAVES MAINTAINED IN A SATURATED ATMOSPHERE FOR 5 DAYS AFTER INOCULATION

Inoculation period	Nos. conidia/drop			
	20	30	40	50
1. No leaf senescence (early April)	53	43	57	55
2. Slight leaf senescence (mid May)	49	87	51	60

(c) Factors affecting formation of aggressive lesions.

(1) Viability of B. tulipae within lesions. After surface sterilizing, Beaumont et al (1936) incubated non-aggressive lesions but without detecting viable B. tulipae. Wilson (1937) was unable to isolate B. fabae from non-aggressive chocolate spot on beans.

To check these negative results, in mid-May 1967, 91 non-aggressive lesions of differing ages were cultured on malt agar (pH 5.0) after being surface sterilized with hypochlorite solution (1.5% Cl_2). When observed 4 days later, B. tulipae had grown from 75 lesions (i.e., 82% of the total) indicating that the pathogen can remain viable within non-aggressive lesions. But for how long is the pathogen viable? Non-aggressive lesions of known age derived from inoculation experiments were cultured at intervals; B. tulipae was isolated from 29 day-old lesions but unfortunately insufficient lesions were available for later observations. Additional trials suggest that the earlier failures of other workers to isolate B. tulipae from lesions might be attributable to excessive exposure to surface sterilants.

(2) Inoculum concentration. The effects of increasing inoculum concentrations on lesion development are shown in Table 21.A where half-leaves were each inoculated at 24 sites. Numbers of non-aggressive lesions did not increase after 48 hr, but when the saturated atmosphere was maintained for longer periods aggressive lesions appeared 4 - 5 days later (Table 21.B). When plotting numbers of aggressive lesions against inoculum concentrations there seemed in this experiment, to be a minumum threshold concentration. Only 1 of 61 lesions caused by 25 conidia/drop became aggressive whereas 61-71% of those caused by 50-200 conidia/drop changed from being non-aggressive, suggesting the existence of a threshold value

Table 21

EFFECTS OF 4 DIFFERENT CONIDIAL CONCENTRATIONS ON NUMBERS OF NON-AGGRESSIVE LESIONS DEVELOPING ON LEAVES WHEN INCUBATED FOR 2 DAYS AND ALSO THE EFFECTS OF PROLONGED INCUBATION ON NUMBERS OF LESIONS, INITIALLY NON-AGGRESSIVE, BUT BECOMING AGGRESSIVE

(24 drops/½ leaf, each 0.002 ml)

	No. conidia/drop			
	25	50	140	200
	Nos. of non-aggressive lesions			
A. 2 days incubation (lesions counted Day 2)				
Replicates	6	13	20	24
	21	24	24	23
	16	21	21	22
	18	19	24	20
TOTAL	61	77	89	89
% successful of 96	64	80	93	93
B. 3 days incubation (lesions counted Day 5)				
Replicates	0	0	18	22
	1	22	15	13
	0	18	14	10
	0	8	16	12
TOTAL	1	48	63	57
% successful of non-aggressive lesions	2	62	71	64

for aggressive lesion formation. The inoculum potentials of many root and soil inhabiting fungi and viruses have been tested, and the work of Wastie (1962) suggests that chocolate spot of beans can be caused by individual conidia of B. fabae. It has already been shown that lesion numbers increase with time and that there is a critical minimum period of saturated atmosphere required before lesions form.

(3) Post-inoculation atmospheric humidity. In testing the effect of extended periods of humid incubation it was found, regardless of concentrations of inocula, that numbers of non-aggressive lesions increased greatly during the first two days with smaller increases on the third (Table 22a). When numbers of lesions formed daily are expressed as percentages, the association with time is clear (Table 22b).

Aggressive lesions first appeared after 3 days incubation with either 40 or 50 conidia/drop concentrations, and by the fifth day they were numerous in all treatments ranging from 20-40 conidia/drop (Table 23). These results parallel those from tests of non-aggressive lesion formation and it seems that all lesions are potentially aggressive providing high humidities and/or water films persist. The indication of a threshold concentration of conidia for aggressive lesion formation detected in experiments testing concentrations of inocula may be attributable to an interaction between this factor and that of duration of high humidity.

(d) Sporulation on aggressive lesions. Field observations and laboratory experiments showed that B. tulipae does not sporulate on non-aggressive lesions and that periods of high humidity and/or persistent water films are a prerequisite for aggressive lesion formation. Are they also essential for the sporulation of

Table 22 a

NUMBERS OF NON-AGGRESSIVE LESIONS DEVELOPING WITH 4 DIFFERENT CONIDIAL CONCENTRATIONS ON LEAVES OF 8 PLANTS INCUBATED IN SATURATED ATMOSPHERES

(15 drops/½ leaf, each 0.002 ml)

Period of incubation		Nos. conidia/drop			
		20	30	40	50
1 day	Replicates	6	8	12	8
		2	2	10	11
		1	10	9	12
		4	7	8	8
	TOTAL	13	27	39	39
2 days	Replicates	8	10	14	14
		5	10	12	15
		8	12	11	13
		8	10	14	10
	TOTAL	29	42	51	52
3 days	Replicates	10	12	14	14
		9	12	14	15
		8	13	12	13
		9	10	14	11
	TOTAL	36	47	54	53

Table 22 b

EFFECTS OF DIFFERENT INOCULUM CONCENTRATION AND PERIODS OF INCUBATION ON NUMBERS OF DEVELOPING NON-AGGRESSIVE LESIONS EXPRESSED AS PERCENTAGES

	Nos. conidia/drop				
	20	30	40	50	Mean
Incubation period	% non-aggressive lesions				
1 day	22	45	65	65	49
2 days	48	70	85	87	72
3 days	60	78	90	87	79

Table 23

NUMBERS OF AGGRESSIVE LESIONS FORMED BY 4 DIFFERENT INOCULUM CONCENTRATIONS 3 AND 5 DAYS AFTER INOCULATION AND MAINTAINED IN A SATURATED ATMOSPHERE

(Total of 4 replicates)

	Nos. conidia/drop			
Period of incubation	20	30	40	50
3 days	0	1	4	4
5 days	19	20	31	29
	Nos. aggressive as % non-aggressive lesions			
3 days	0	2	7	9
5 days	53	43	57	55

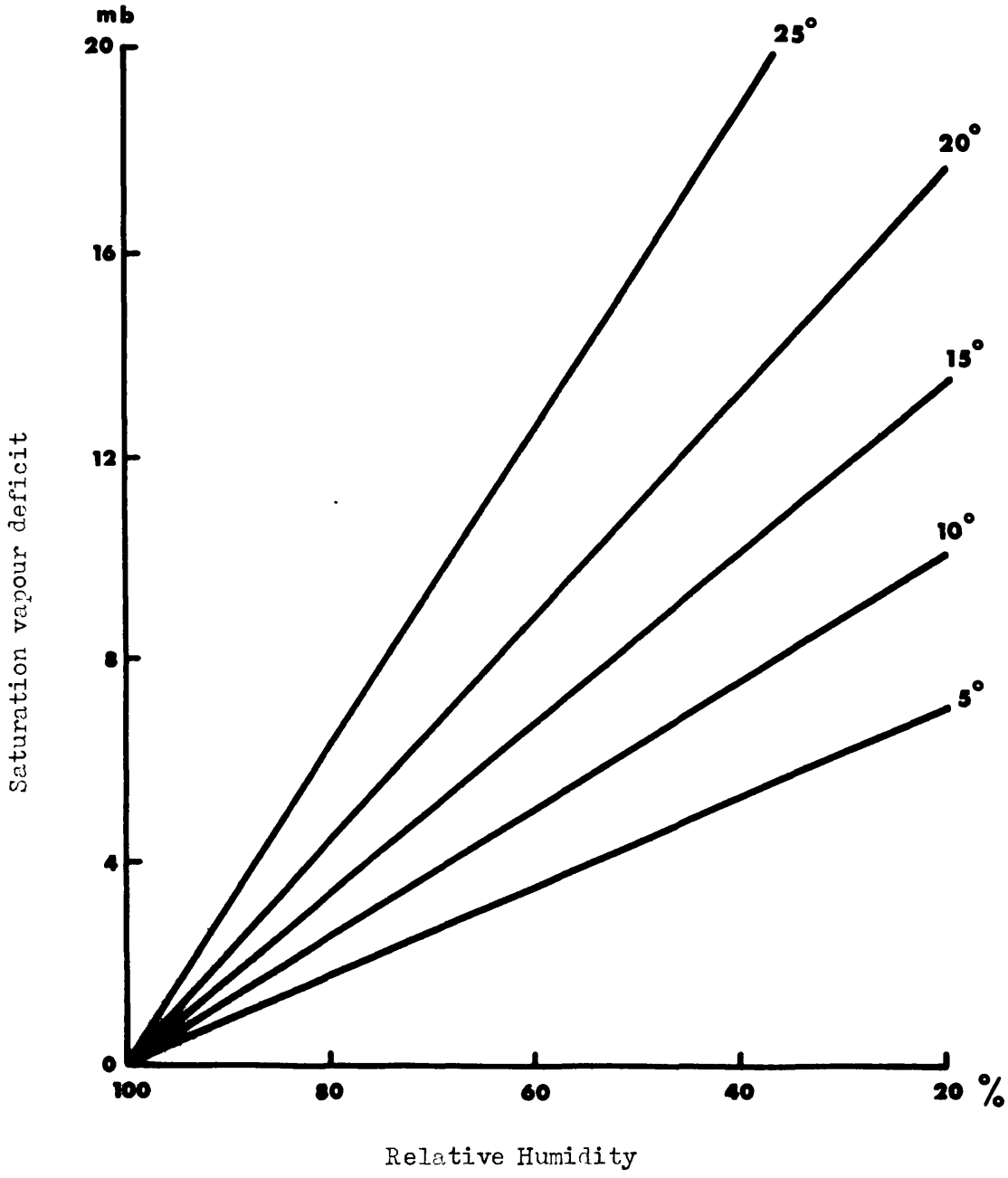
B. tulipae within these lesions? In an attempt to determine the factors influencing sporulation, aggressive lesions were formed on leaf discs, cut out with cork borers, in chambers with differing humidities using Jarvis's apparatus (1960), where the discs could be moved from one humidity regime to another with little disturbance. Because temperature changes exert a large effect on relative humidities, discs were constantly at 18°C and the humidities were expressed in millibars of Saturation Vapour Deficit. The differing regimes were obtained using glycerine/water mixtures which are not sensitive to small changes of temperature (Carson 1931). The relationship between absolute humidities expressed as Saturation Vapour Deficit, and Relative Humidities at five temperatures is shown in Fig. 4. In choosing the three humidities - 0.0, 1.7 and 3.5 mb, equivalent to 100, 90 and 80% Relative Humidity at 15.5°C respectively - heed was taken of Jarvis's results (1962a) comparing effects of different Relative Humidities on B. cinerea sporulation.

B. tulipae developed in infection drops on the discs to the following extent after 4 days incubation:

1. 0.0 mb S.V.D. (saturated atmosphere).
   Dense hyphal growth in and out of spore droplet with lesion extending beyond droplet circumference.
2. 1.7 mb S.V.D.
   As for 0.0 mb.
3. 3.5 mb S.V.D.
   Leaf disc shrivelled, hyphae covering lesion and abundant conidial production.

After making the above observations the leaf discs were moved to different humidities and reincubated for a further 3 days and

Fig. 4. Conversion of atmospheric humidities (%) to saturation vapour deficits (mb) - a measure of atmospheric humidity.



re-examined.

4. 0.0 mb - 0.0 mb (continuously in saturated atmosphere).

   Hyphal mass slimy, no sporulation.

5. 0.0 mb - 1.7 mb

   Hyphal tuft over lesion, no sporulation.

6. 0.0 mb - 3.5 mb

   Hyphal tuft over lesion, leaf disc shrivelling, no sporulation.

7. 1.7 mb - 0.0 mb

   Hyphae growing over whole leaf disc, no sporulation.

8. 3.5 mb - 1.7 mb

   Leaf disc shrivelled, abundant sporulation.

These results, confirmed in series of replicate experiments, show that high humidities favour superficial mycelial growth and prevent sporulation. Conidial production occurred at the lowest humidity tested which tended to dessicate leaf tissues. Supporting field experimentation is required, but these results suggest that sporulation depends more upon external conditions than those within leaves and that conditions for the successful natural propagation of fire include:

(1) water drops to disperse large enough numbers of conidia to form aggressive lesions, and

(2) drying conditions after aggressive lesion formation to allow sporulation.

This observation is seemingly in conflict with records made of numbers of airborne Botrytis conidia (Hirst 1953). Hirst found that concentrations were maximal in dry conditions but a distinction needs to be drawn between absolute numbers and effective numbers in etiology. My experiments suggest that conidial

aggregates are required for aggressive lesion formation and these are more likely to be transferred from their primary sources by splash drops than by convection eddies - an observation in line with those of Jarvis (1962b) on the dispersal of B. cinerea in soft fruit plantations.

(e) Daughter bulb infection and conidia. Although conidial dispersal, by wind and water, to other leaves causes yield-decreasing lesions, conidia might also infect developing daughter bulbs, so possibly aiding disease perennation. In the field, the incessant plant movement tends to compact soil such that a tube forms which is slightly larger than the stem so forming a channel in which water trickles unimpeded from leaves down stems to the necks of mother and developing daughter bulbs. If conidia were carried in suspension this could form an infection pathway similar to that described by Lacey with potatoes and Phytophthora infestans (Mont) de Bary (1962). To test this hypothesis, 20 ml aliquots of conidial suspension were poured at fortnightly intervals in May and June down Rose Copland tulip stems growing in ridges. These, and 20 uninoculated plants were lifted in early July and examined for symptoms of fire below ground. The results (Table 24) were inconclusive but critical work using bulbs systematically planted in different attitudes might be rewarding, especially if the investigation were linked with a study of soil moisture. To gain some idea of soil tension, measurements of 18 tensiometers placed at bulb depth in a ridge planted with tulips were made throughout the summer. Full results are given in Appendix 1. The weekly mean values expressed as log. tension in cm H_2O as suggested by Rutter (1964), show a gradual but erratic reduction in soil moisture. This drying of the soil would influence the extent to which conidia in

dew or rain would penetrate down the flower stalk - dry conditions making daughter bulb infection less probable than wet.

Table 24

NUMBERS OF STEM LESIONS FORMED WHEN CONIDIAL SUSPENSIONS OF *B. tulipae* WERE POURED DOWN STEMS OF ROSE COPLAND TULIPS GROWING IN RIDGES

Date inoculated	No. of inoculated stems	No. of diseased stems when examined in early July
16 May	18	3
6 June	19	2
20 June	18	2
Uninoculated control	20	2

B. Tulip phylloplane non-parasitic micro-organisms.

The ecology of leaf inhabiting micro-organisms has been reviewed by Last and Deighton, 1965; Sinha, 1966; and Last and Price, 1969, who indicated that too little attention had been paid in the past to the possible effects of leaf saprophytes on the pathogenesis of leaf parasites. Ruinen (1966) showed that *Cryptococcus laurentii* (Kufferath) Skinner and *Rhodotorula glutinis* (Fres.) Harrison produce lipolytic enzymes that erode leaf surfaces so making leaves more wettable. This changing property may favour the germination and entry of leaf pathogens. The erosion of leaf

surfaces also increases the loss of water by transpiration through the leaf; any increase in water loss possibly hastens senescence or abscission of leaves such as occurs in Hevea brasilliensis L. attacked by Oidium heveae Steinmann.

Tulip leaves are water repellent when first unfurled because of superficial wax-like cylinders but as the season progresses these are worn away either by friction, leaves brushing against their neighbours, or by microbial activity. In the 1966-67 spraying experiment at Rosewarne E.H.S. (vide Appendix 9) leaf senescence was delayed in mancozeb sprayed plots but not in dichofluanid or unsprayed plots, although in that year fire was absent. This delayed senescence was later associated with significantly greater yields than those from the earlier senescing treatments. Bruinsma (1964) suggested that this greater longevity of leaves is associated with biochemical activities within the leaves, but it seems equally possible that the effect might be attributable to the decreased activity of naturally occurring 'saprophytes'. Evidence has been put forward by Toussaint (1968) showing that sprinkle irrigation of tulips after flowering delayed senescence, which is not inconsistent with the suggestion that leaf inhabiting organisms shorten leaf life by increasing transpriation when eroding cutin.

The nature and frequency of populations of phylloplane micro-organisms were determined on mancozeb sprayed and unsprayed tulip leaves at intervals throughout the growing season. The dilution plate method, although used initially, was considered inappropriate because it favours fungi which readily sporulate or fragment, and the propagule counts overestimated numbers of leaf colonies of such fungi as Penicillium species. Instead leaf discs

Plate 5.

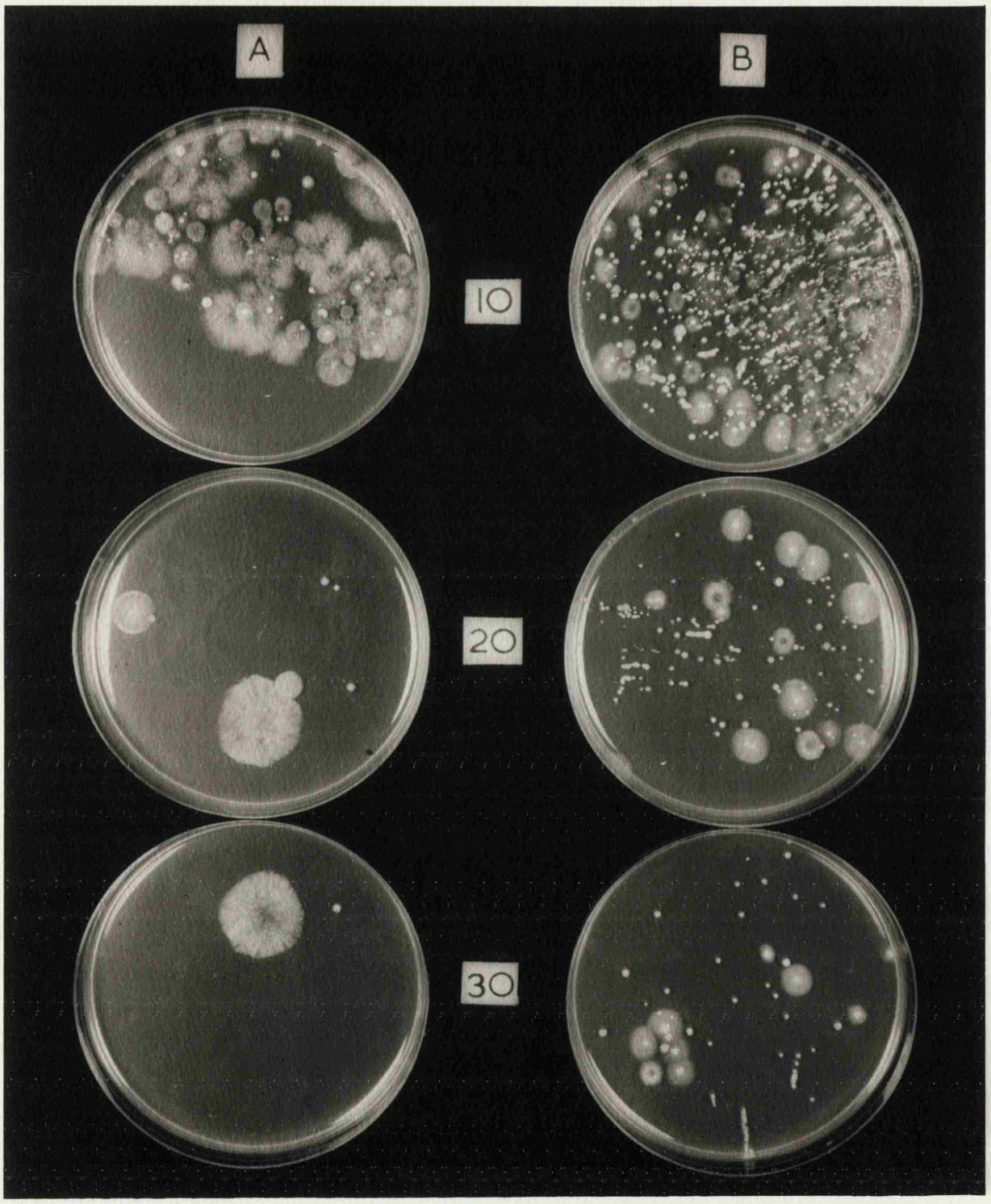


Plate 5. Colonies developing from (a) sprayed and (b) unsprayed leaves after 10, 20 and 30 minutes agitated washing, incubated at 20°C for 4 days.

were shaken for a total of 60 min, but with changes of water every 10 min to provide six sets of washings per sample. It was assumed that the profusely sporing species of fungi and casual inhabitants caused high colony counts in the first set but subsequent values were more representative of the residents of this ecological niche. Instead of mixing suspensions with agar before gelling, which might reduce the development of aerobes, 0.2 ml were pipetted on to the surface of solid media to encourage their greater development. Media used included acidified malt agar (pH 5.0), malt agar and aureomycin, malt agar and sodium proprionate, and nutrient agar (pH 7.0). The presence of ballistospored yeasts was checked using the spore-fall method described by Buller (1933). Three replicate petri dishes of each media were used for each set of washings (Table 25).

More colonies developed from the first set of washings than from subsequent lots. Thus from unsprayed leaves sampled on 15 May, an average of 599 colonies developed per 10 cm^2 of leaf from the first washing declining in subsequent equal periods of washing to 31, 7, 3, 3, and 0 linearily. On the sprayed plants there was an indication of seasonal build-up, there being fewer micro-organisms in early May than later. Spraying decreased numbers of developing colonies from 53 and 20 to 1 and 0.4 per 10 cm^2 in the fourth and fifth washings of leaves sampled on 24 June. (Plate 5).

After incubating for four days at 15°C, it was possible to distinguish between filamentous fungi, yeast-like organisms and bacteria. Comparative figures (Table 26) show that leaf microflora consisted mainly of yeasts, although by late June numbers of filamentous fungi were increasing and those of yeasts decreasing. Micro-organisms found and identified as phylloplane inhabitants

Table 25

NUMBERS OF MICRO-ORGANISMS PER 10 CM^2 DEVELOPING FROM A SUCCESSION OF 6 WASHINGS WHEN PLATED ON ACIDIFIED MALT AGAR

(Means of 3 petri dishes/sequence)

Sequence of washing	Date of sampling				
	7/5	15/5	20/5	3/6	24/6
	Nos. colonies after 4 days incubation at 25°C				
1. Sprayed plants					
1	1.1	-	85.4	29.7	54.4
2	1.3	-	5.9	3.8	4.0
3	0.2	-	0.8	1.3	1.7
4	0.0	-	-	-	0.8
5	0.8	-	-	-	0.4
6	0.2	-	-	-	0.0
2. Unsprayed plants					
1	-	599.0	621.0	∝	∝
2	-	31.4	41.8	∝	74.0
3	-	6.6	16.8	79.0	53.9
4	-	2.9	-	-	52.6
5	-	2.8	-	-	19.6
6	-	0.0	-	-	16.4

are given in Table 27.

The paucity of bacteria could be attributed to an artefact of the isolation technique although the phylloplane might be unfavourable for their development, water drops accumulating in leaf axils becoming acid pH 5.0. In general, organisms per unit area were few compared with numbers found on other leaves at this time of the year, including barley (Last, 1955), potato (Holloman, 1967), and apples (Marshall and Walkely, 1951).

This study of the tulip phylloplane indicates that B. tulipae grows in an environment colonised by other micro-organisms which may influence its development.

The tulip phylloplane investigation is brief because results are based on one season's uncorroborated data.

Table 26

COMPOSITION OF MICRO-FLORAS ISOLATED FROM SPRAYED AND UNSPRAYED TULIP LEAVES SAMPLED ON 3 OCCASIONS.

(Means of 5 petri dishes/sample)

Treatment	Sampling date	Nos. of colonies /cm^2 leaf				
		Filamentous fungi	Yeasts		Actino-mycetes	Bacteria
			white	pink		
Mancozeb	20/5	0.2	5.6	0.0	0.2	0.0
sprayed	2/6	0.2	3.4	0.0	0.0	0.0
plants	24/6	1.7	1.7	0.6	0.0	0.0
Unsprayed	20/5	0.8	15.7	0.2	0.0	0.0
controls	2/6	0.8	77.4	1.1	0.0	0.0
	24/6	16.6	31.2	6.2	0.0	0.0

The disparities between totals in this table and Table 25 are caused by rounding off values.

N.B. Counts refer to populations in third washing (unsprayed plants) and second washing (sprayed plants), because colonies on one of the samples of sprayed and unsprayed plants of the same sequence were either too numerous or too few.

Table 27

MICRO-ORGANISMS FOUND DURING THE SPRING AND SUMMER ON SURFACES OF SPRAYED AND UNSPRAYED TULIP LEAVES IN SUSSEX, 1968.

Mancozeb sprayed plants

Aureobasidium pullulans (de Bary) Arn.

Botrytis tulipae

Candida albicans

Sporobolomyces roseus Kluyver et v. Niel.

Yeast sp. (white)

Yeast sp. (cream)

Unsprayed controls

Acrostalagmus cinnebarinus Corda.

Aureobasidium pullulans

Botrytis tulipae

Candida albicans

Chaetomium spp (2)

Cladosporium herbarum Fr.

Humicola sp.

Phoma sp.

Sporobolomyces roseus

Tilletiopsis washingtonensis Derx.

Trichoderma viride Pers.

Yeast sp. (white)

Yeast sp. (cream)

BELOW-GROUND ETIOLOGY OF TULIP FIRE

(a) Field observations. When the life cycle of fire was considered by Hopkins in 1921 and by Beaumont et al in 1935, their attention was focused more on the above-ground symptoms than below-ground aspects of the disease, i.e. the appearance of primaries and subsequent leaf-spotting.

What happens to the fungus between bulb-lifting in July and its appearance in the field on primaries in the following spring? Hopkins (1921) detected B. tulipae sclerotia in soil and on bulbs, and as mycelial lesions on fleshy scales, but he assumed that the fungus did not commence growth until spring. He also noticed that infected shoots were associated with neck-infected bulbs. Beaumont et al (1936) stated that fire lesions on fleshy scales always contained sclerotia, implying that lesions without sclerotia were not attributable to B. tulipae. An example was quoted where only four of a thousand bulbs from a severely infected crop were found with sclerotia, and they observed that the removal of old scales, flower stems and roots, prior to grading, would remove much of the pathogen-bearing material. The impression given at the time, was that sclerotia in the soil from previous tulip crops presented a serious threat.

My observations on samples of commercial bulbs differ considerably from those recorded by previous investigators. In the autumn of 1966, disease observations were made on William Pitt bulbs sent from Kirton.

Of 540 bulbs - 313 were without lesions,
16 had fire lesions with sclerotia,
73 had fire lesions without sclerotia and
138 had lesions not attributable to B. tulipae.

Plate 6.

Plate 6a. 'Split base' - a symptom associated with root plates attacked by B.tulipae.

Plate 6b. Appearence of mother bulbs with 'split base' planted in previous October, showing abnormal daughter development in May.

Sclerotia developed on only 18% of the bulbs with B. tulipae, all of which yielded B. tulipae when surface sterilised (hypochlorite) pieces of diseased tissue were cultured on malt agar slopes. The lesions not attributable to B. tulipae yielded species of Penicillium and Trichoderma, which probably secondarily invaded damaged tissue. But because these bulbs were derived from a spraying experiment, they could not be considered to be a truly representative commercial sample. In addition to the symptoms already noted some bulbs of the Darwin Hybrid group, including Apeldoorn, Oxford, Dover and London, have been found recently with split root-plates ('split-base' see Plate 6), an effect associated with colonising B. tulipae (Appendix 2). It seems possible that splitting occurs if the pathogen attacks the stem base when the root plates of the daughter bulbs are enlarging.

In 1967, 3,000 stored bulbs (Oxford), which had been sprayed regularly in the previous season and grown on land previously uncropped with tulips, were examined. In this sample sclerotia were more abundant on superficial tunics and less common on lesions on fleshy scales, (Table 28). Further, B. tulipae was commoner on bulbs than previous investigators had indicated. It has been shown in several soil-borne diseases that the nearer the inoculum to the host the more likely the infection (Coley-Smith, 1960; Fellows and Ficke, 1934), and if this is true for B. tulipae, these bulb infections present a greater threat to the subsequent development of bulbs than sclerotia in the soil from previous tulip crops, especially as crop rotation, frequently as long as 6 years, is de rigeur amongst bulb growers.

What causes a primary? Clearly the bulb is never completely dormant: but is the fungus active in the lesion

Table 28

TYPE OF *B. tulipae* INFECTION ON A COMMERCIAL SAMPLE OF STORED OXFORD TULIP BULBS EXAMINED AT PLANTING

(Nos. infected of 3,000)

Type of infection	Nos. within each category	% total of bulbs infected with *B. tulipae*
'Split base'	44	21.0
Sclerotia on tunic and/or remains of previous seasons flower stalk but not on fleshy scale	131	64.0
Sclerotia *not* on tunic but on fleshy scale	1	0.5
No sclerotia, fleshy scale lesion only	25	12.0
Sclerotia on tunic and fleshy scale	5	2.5
TOTAL	206	

during bulb storage or is it dormant? If B. tulipae remains dormant, what activates it?

An experiment comparing the respiration rates of previously infected bulbs with lesions measuring 13 cm dia. and healthy bulbs during storage showed that the presence of B. tulipae did not affect respiration, suggesting that the fungus was dormant (Appendix 3).

Bulb inspections prior to planting have shown that sclerotia and lesions occur anywhere on the outer surfaces of fleshy scales. Could the position of these affect the subsequent development of the pathogen? Hopkins (1921) associated with primaries with bulb infections in the neck region. In March 1966, 237 bulbs with diseased foliage were rogued as primaries from a crop on land which was being cultivated with tulips for the first time (Table 29). These bulbs were rogued from c. 200,000 (the number necessary to plant an acre) and because the land had not been cropped with tulips before it is almost certain that the disease was carried on the newly acquired stock of bulbs. In 11 of 237 instances (5%) the foliage was diseased but the bulb healthy, indicating that secondary infection had taken place in March. Re-arranging the figures for primaries growing from diseased bulbs indicates that 98% of the primaries were infected at the neck, 73% at the middle and only 34% at the base, confirming the observations of Hopkins.

A second batch of roguings received two weeks later were assessed for symptoms of fire, bulbs being cut longitudinally to see the extent of internal lesions (Table 30). These bulbs had been planted some 6 - 7 months previously, and if it is assumed that the original site of infection was superficial or external, the data suggest that the fungus penetrates into bulbs as well

as superficially down the outside of the first fleshy scale. The fungus grew in and on the inner and outer surfaces of the fleshy scales rather than through them. If then, the primaries are associated with neck infections, what effects do infections elsewhere have on the bulb? Would the pattern of disease vary between similarly infected bulbs planted in beds and ridges?

Table 29

NUMBERS, POSITION AND EXTENT OF EXTERNAL INFECTION OF BULBS ROGUED AS PRIMARIES ON A COMMERCIAL FARM IN MARCH 1967.

Type of lesion	Nos. of bulbs within each category
(1) Primaries	
Neck region only of outer scale infected	58
Lesion extending from neck to midway down outer scale	87
Lesion extending from neck to base of outer scale	76
Lesion at middle of outer scale	1
Lesion extending from middle to base of outer scale	1
Lesion at base of outer scale	3
(2) Secondaries	
Shoot diseased, bulb healthy	11
TOTAL	237

Plate 7.

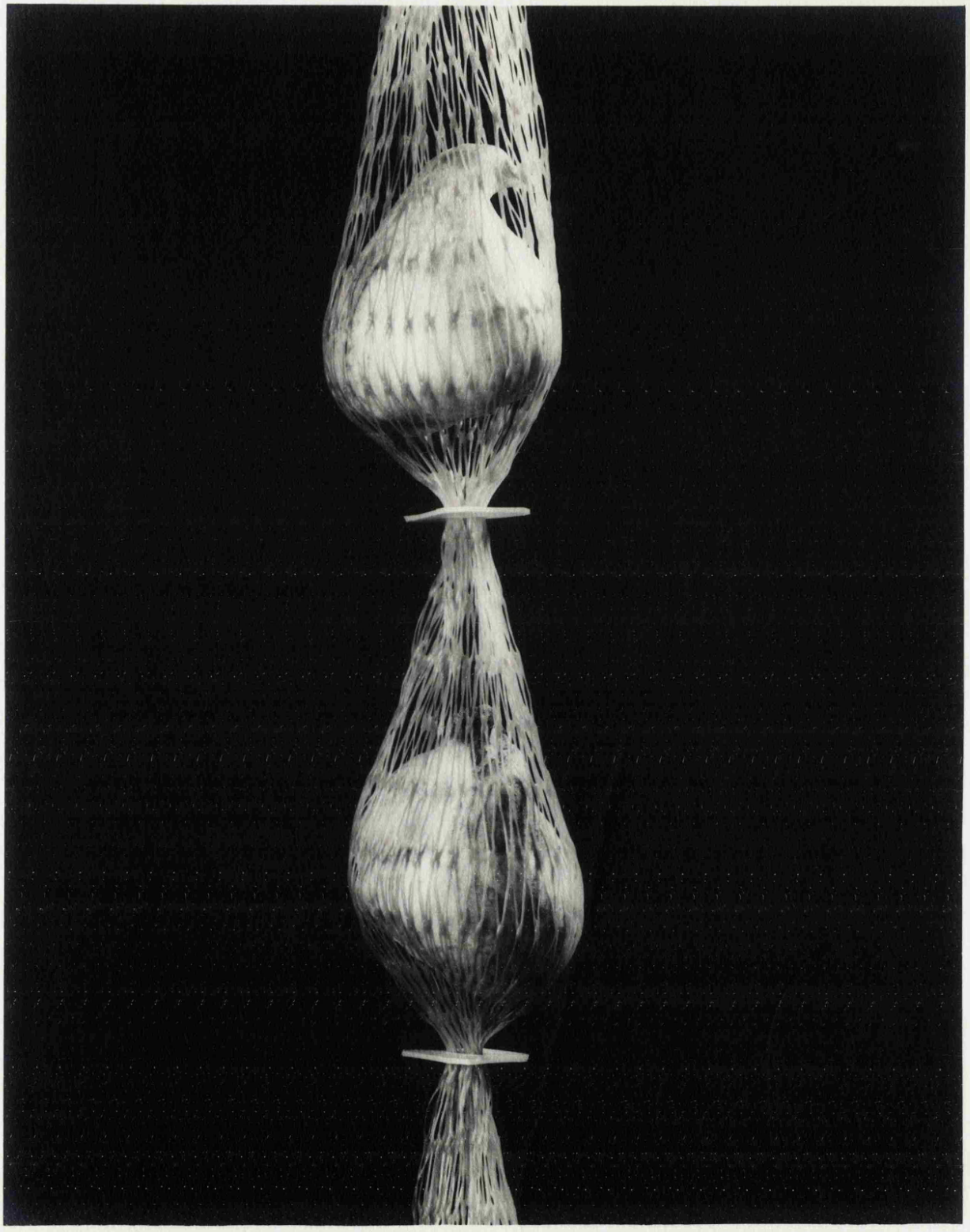


Plate 7. Bulbs placed in 'Netlon' to facilitate retrieval.

Table 30

NUMBER, POSITION AND EXTENT OF INTERNAL INFECTION OF BULBS ROGUED AS PRIMARIES ON A COMMERCIAL FARM IN APRIL 1967

Location of lesion	Scales infected			
	1st	1st/2nd	1st/2nd/3rd	Total
Neck region	11	23	53	87
Neck to middle	11	18	48	77
Neck to base	3	7	27	37
TOTALS	25	48	128	201

(b) Inoculation experiments.

(1) Comparison of position of inoculation and planting methods. To examine these factors an experiment was done using artificially infected bulbs. Bulbs (10 cm Rose Copland) were arranged in plastic nets ('Netlon') being spaced five inches apart and isolated from each other by small plastic clips (Plate 7). This method was developed at Rosewarne E.H.S. in 1965 which makes lifting easy in adverse weather and also makes it possible to trace the fate of tagged bulbs. For convenience, ten bulbs were placed in each net and inoculated with a small piece of dried agar containing several sclerotia of B. tulipae. Inocula were prepared by incubating malt agar cultures in the dark at 20°C, drying them after sclerotia formation, which usually occurred after two weeks. The dried cultures were then inserted into oblique cuts made with a scalpel in the outer fleshy scales. A few days after being

inoculated small lesions developed (at the end of August). The bulbs were planted in late October (Appendix 4) and two mercury reservoirs of a continuously recording thermograph were buried at bulb depth - one in a bed and the other in a ridge. Each week temperatures were read at 2 hr intervals and from them maximal, minimal and modal temperatures were deduced (Fig. 5). Although temperature fluctuated over a wide range it was at no time below freezing point nor did it drop below the lower cardinal point for the in vitro growth of B. tulipae.

Every fortnight, from late November onwards, ten bulbs of each inoculation treatment and controls were lifted and examined for fire, shoot length being measured until they emerged above ground. Mean logarithmic values of shoot length in 1965/66 and 1966/67 are plotted against time in Fig. 6. Although shoots began to extend sooner, possibly because of the earlier planting in 1967 than in 1966, the linearity of logarithmic growth rates agree with results reported by Rees (1968) working with forced tulips. The arithmetic values for shoot elongation are compared with weekly modal soil temperatures in Fig. 7, and it seems that, once started, the emergence rate is, in general terms, independent of weekly changes in soil temperature.

Previous investigators of tulip fire have tended to consider different aspects of the disease as unrelated entities - leaf symptoms, bulb symptoms, etc., and this has had the unfortunate effect of making the relationship between host and pathogen appear discontinuous, instead of a series of closely related happenings. The fortnightly evaluation of the progress of disease showed aspects of this relationship which had previously passed unnoticed. The development of tulips is conveniently divisible into three

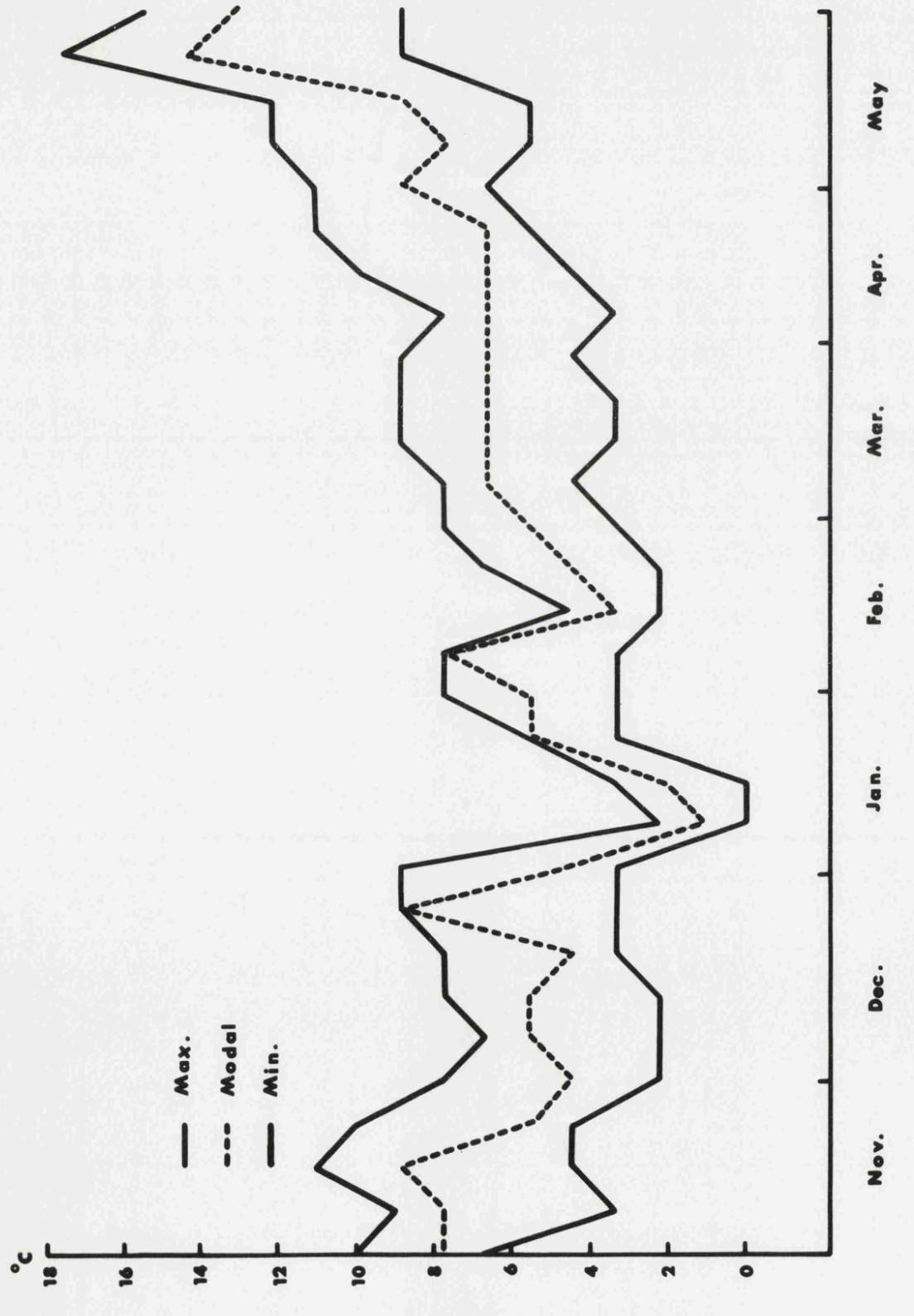


Fig. 5. Soil temperatures recorded in tulip ridges at bulb depth, 1966/67.

Fig. 6. Rate of shoot elongation below ground by tulips grown in ridges. 1966/67 ———— and 1967/68 ----------.

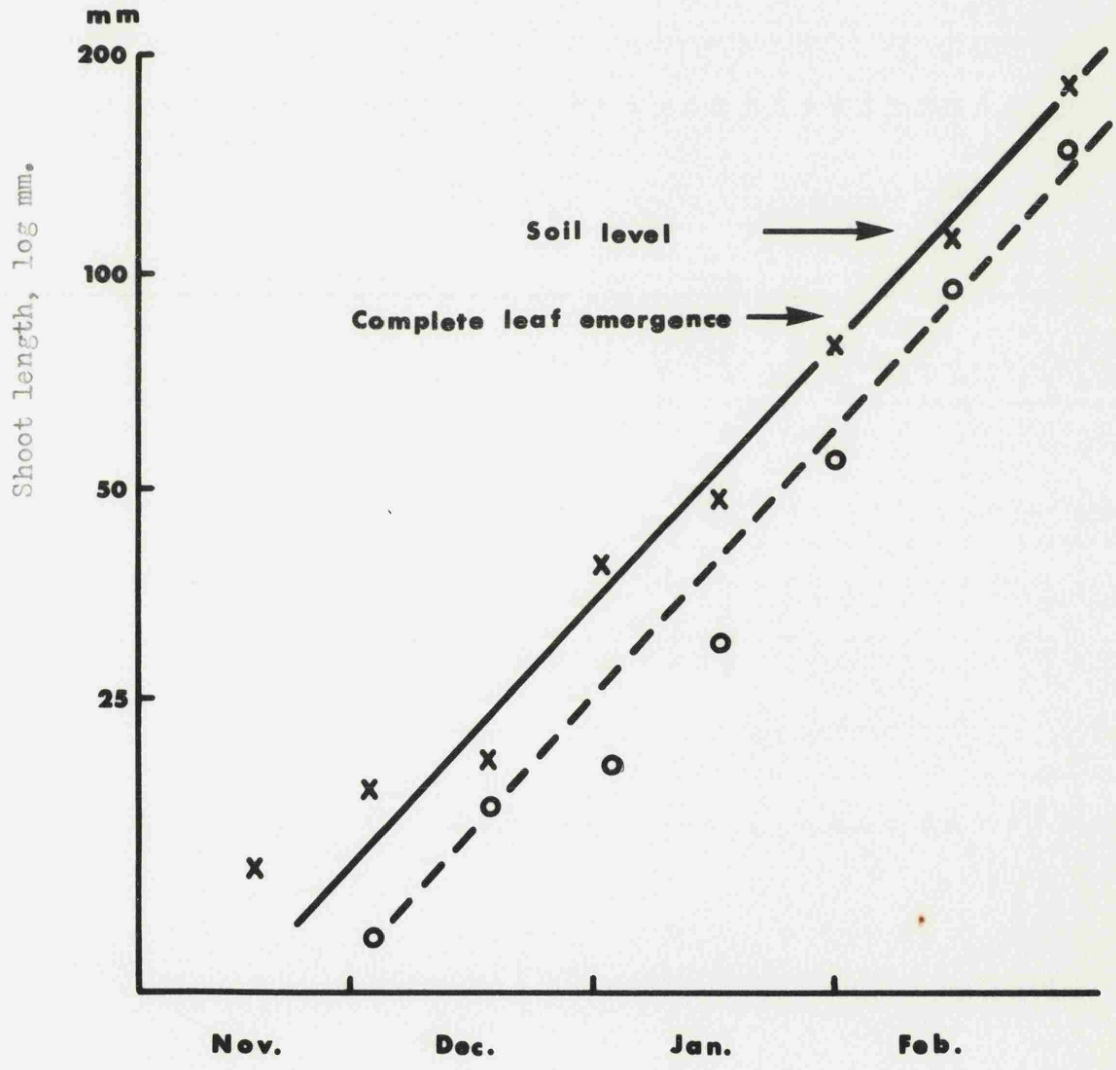


Fig. 7. Comparison of tulip shoot elongation and modal soil temperature in ridges.

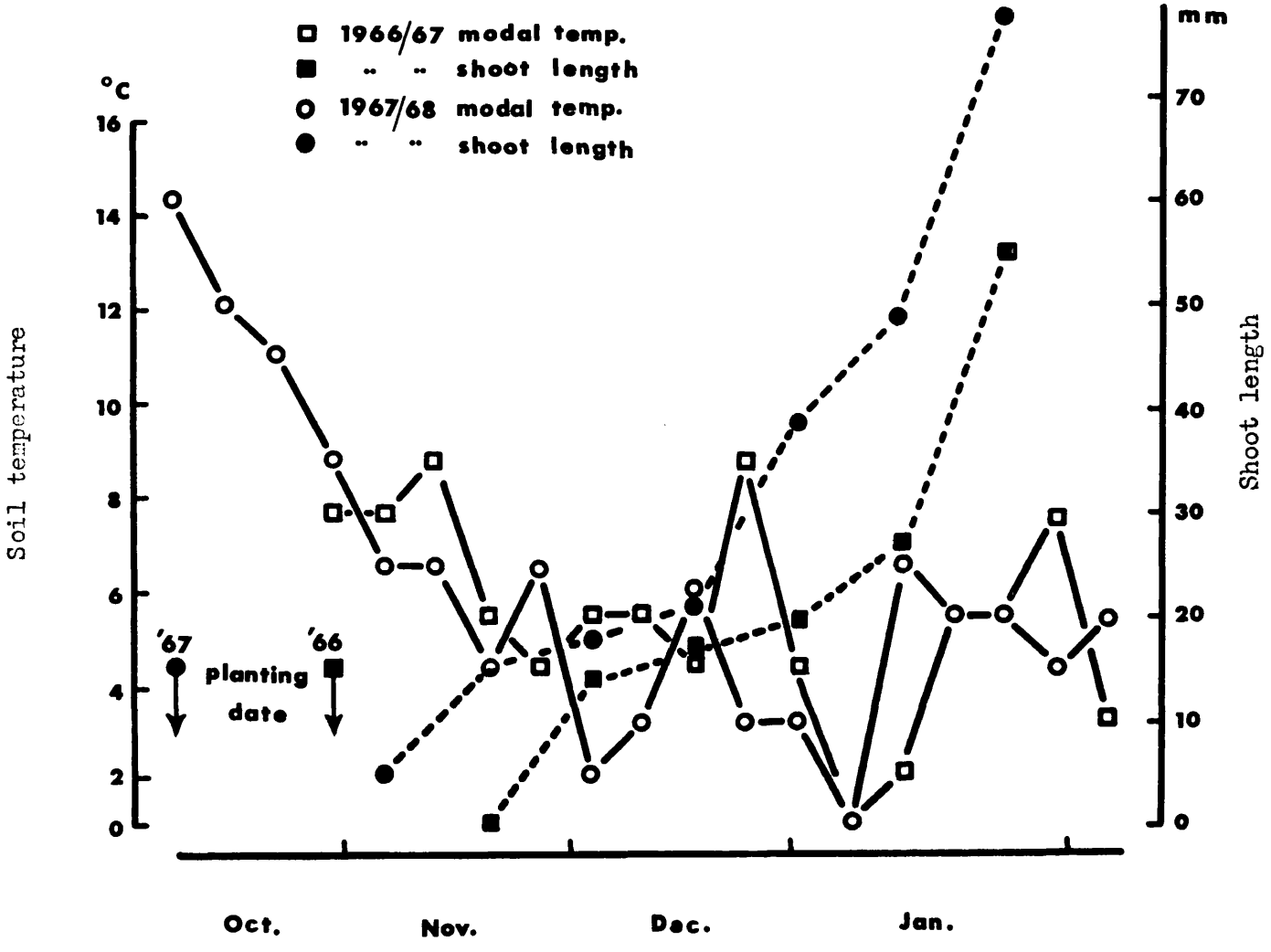


Plate 8.

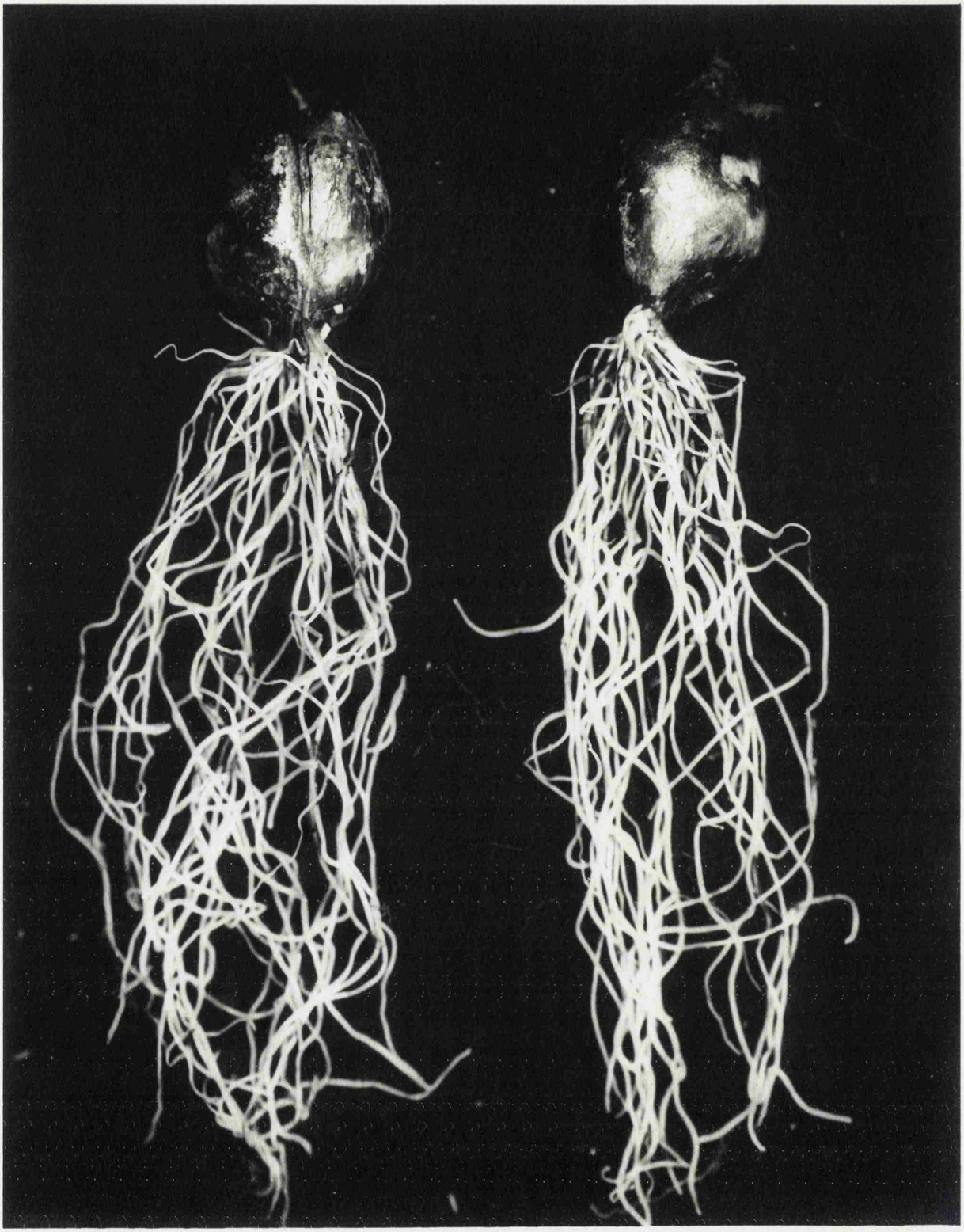


Plate 8. Bulbs completely rotted by B.tulipae subsequent to rooting.

parts:

(1) from commencement of growth to shoot emergence above ground,

(2) from shoot emergence to heading and from

(3) heading to leaf senescence.

Heading is the name given to the cultural operation when, shortly after the flowers are seen to be true to type, they are snapped off.

The samples of bulbs lifted every fortnight were examined and placed in one of five categories -

(1) Bulbs completely rotted, rotting occurring after root formation.

(2) Leaves infected.

(3) Stems infected below ground.

(4) Shoot healthy but mother bulb rotting.

(5) Shoots healthy and artificial infections of bulbs not spreading.

The results are expressed in Table 31 as percentages excluding infection in category 5; the full numerical data are given in Appendix 4.

As the experiment progressed it was recognised that attack followed several different pathways causing different sets of symptoms which could vary considerably (Fig. 8), with only some being discernible above ground, i.e.,

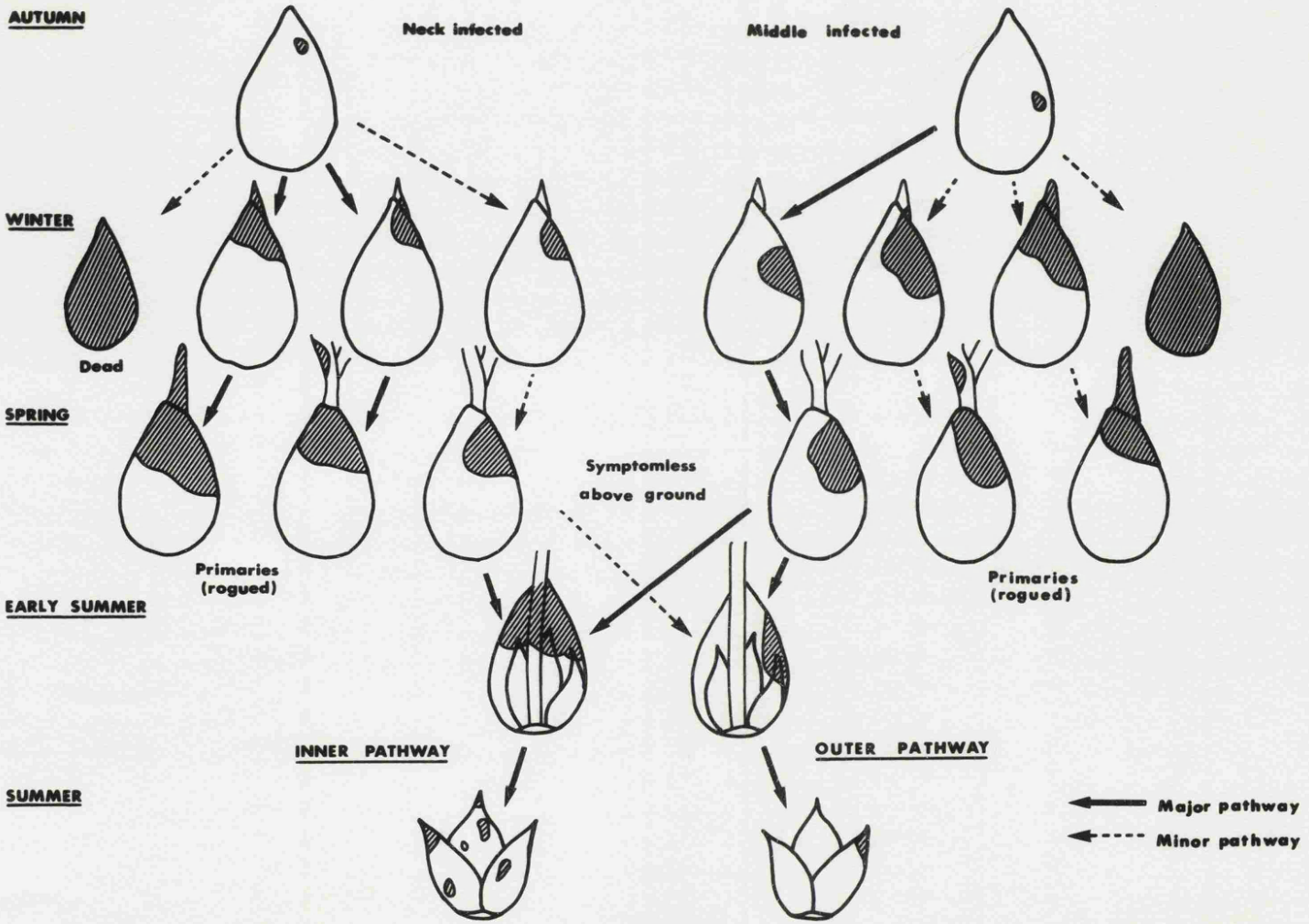
(1) A few plants rotted completely soon after rooting and shoots never appeared above ground (Plate 8). 'Misses' would probably pass unnoticed and in any event would be unreliable indicators except in systematically arranged plantings. They do, however, lead to contamination of soil with sclerotia.

Table 31

EFFECTS OF DIFFERENT SITES OF INOCULATION AND CULTURAL PRACTICES ON THE DEVELOPMENT OF TULIP FIRE (%)

		Distribution of infection		
		Shoot not emerging	Shoot emerging	
		Mother bulb rotted	Mother bulb rots	
			Shoot healthy	Shoot infected
				Stem / Leaf
(a) Changes in expression of disease during the growing season				
Sampling period				
Planting - emergence	1	76	0	23
Emergence - heading	0	70	10	20
Heading - late May	2	70	15	13
Seasonal value	1	72	9	18
(b) Mean effects of different sites of inoculation				
Middle of bulb	2	85	7	6
Neck of bulb	2	56	10	32
(c) Mean effects of different planting procedures				
Bulbs planted in beds	1	76	10	11
Bulbs planted in ridges	1	66	8	25
(d) Interaction between different sites of inoculation and planting procedures				
Bed planted, middle lesion	2	84	7	7
Bed planted, neck lesion	1	70	12	17
Ridge planted, middle lesion	1	86	7	6
Ridge planted, neck lesion	2	44	9	45

Fig. 8. Diagrammatic representation of infection pathways of B. tulipae in tulip bulbs which result in the perennation of tulip fire.



Plates 9 & 10.

Plate 9.

A potential primary before emergence above ground. Shoot infected in December, photographed in January.

Plate 10.

First leaf primary in March.

(2) (a) Primaries are formed when developing shoots sometimes become infected as they pass through the diseased neck of bulbs. If infection occurs at an early stage whole shoots may rot, but not before appearing above ground as extension growth proceeded (Plate 9).

(b) A less extreme form of attack occurs when the top part of the leaf escapes infection but the lower part is attacked (Plate 10) - to form a previously underrated type of infection. The outer leaf encircling the shoot, the first leaf, lies close to the ground and as a result these lesions are obscured until secondary leaf spotting draws attention to the primary source.

(3) After the foliar parts of the shoot pass through the neck it is possible for the below-ground stem to become infected, lesions subsequently partially or wholly ringing the stems.

(a) If the entire stem is ringed its brittleness increases the chances of it being snapped off in the strong winds prevalent in the flat areas of eastern Britain.

(b) Partial ringing disturbs the metabolism of the plant and leaves appear glossy, lacking wax and being light green in colour.

(4) At a later stage of growth infected tulip bulbs with healthy shoots can continue to rot without above-ground symptoms. The pathogen on the bulbs may spread and affect development of daughter bulbs.

Previous investigators have emphasised the importance of primaries because of resultant secondary leaf spotting, but the mean seasonal values for this experiment indicate that in 72% of

Plates 11 - 13.

Plate 11.

Lesion on outermost daughter of B.tulipae infected mother bulb with healthy shoot.

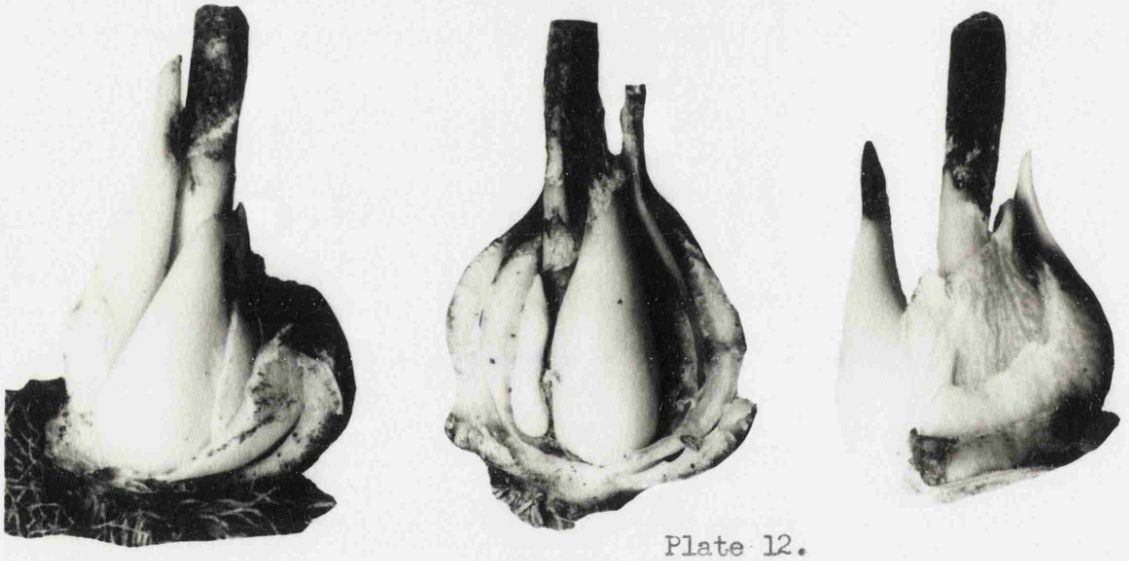


Plate 12.

Innermost daughters infected by contact with diseased fleshy scale and flower stalk.

Plate 13.

Bulb cluster at lifting infected via inner pathway.

of instances B. tulipae remained on underground structures with little effect on shoot development; on only 18% of instances did leaf infection appear. This value of c. 70% was constant throughout the experiment but the proportion of leaf and stem infections among the remaining 30% varied.

When bulbs commenced shoot growth only leaves were exposed but with the continued elongation stems subsequently appeared, with the proportions of leaf infections decreasing from 23% during planting to emergence to a minimum of 13% from heading to late May. Numbers of stem infections increased for two reasons -

(1) the foliar part of the shoot is no longer available for infection, having passed through the neck, and

(2) after flower colour develops (approximately at heading) shoot extension ceases and stems become stationary, making infection more likely, there no longer being a moving target.

The most striking result of this experiment was the discovery of below-ground infection pathways from mother to daughter bulbs. Many of the emerging shoots escaped infection and daughter bulb development was seemingly normal. Enlargement of daughter bulbs, however, brought them nearer the diseased tissues of the mother, and a proportion became infected (Table 32). This infection pathway took place in two ways -

(1) the outermost daughters became infected from the diseased fleshy scales of the mothers (Plate 11) or

(2) the innermost daughters were invaded by B. tulipae spreading from the bases of infected stems and fleshy scale leaves (Plate 12 & 13).

Table 32

THE LATE SEASONAL TRANSFERENCE OF B. tulipae FROM MOTHER BULBS TO DAUGHTER BULBS

Date of sampling			
10 April	24 April	8 May	22 May
2/40*	4/40	21/40	25/40

* Numerator - number of infected clusters
Denominator - number of clusters examined

In their early development, the innermost daughter bulbs are closely pressed to the stems and infection commonly takes place at the daughter bulb's tip. Later, as mother bulb scales disintegrate the daughter bulbs swell and pivot on the base plate away from the stem, infection at this stage occurring further down the daughter bulb. For most of the development period the outermost scales of daughter bulbs are fleshy but soon after the mother plant flowers these scales become membraneous to form the tunic. This change seems to be associated with the increasing size of axillary buds. If infection occurs when outer scales of daughter bulbs are still fleshy the fungus may penetrate deeper to infect the next scale. These symptoms are sometimes masked if the brown and membraneous tunic remains intact. Lesions may occur virtually anywhere on daughter bulbs because of the potential variation of infection allowed by the underground pathway of attack.

Lesions near the neck tend to cause more primaries than

those elsewhere - a result borne out by the experiment (Table 31 b). The data in Table 31 c suggest that ridge planting favours the production of primaries, a result that is inexplicable at the present time. During winter months there was no great difference between ridge and bed soil temperatures (Table 33). Ridge temperatures increased sooner on warm days but cooled sooner so that differentials were either small or cancelled out. The soil moisture from planting until late spring was continuously at field capacity so differences of disease expression are unlikely to be attributed to difference of temperature or soil moisture content.

Table 33

COMPARISON OF WINTER SOIL TEMPERATURE (°C) AT BULB DEPTH IN BEDS AND RIDGES - 1966/67

Week ending	Ridge			Bed		
	Max.	Modal	Min.	Max.	Modal	Min.
7 November	10.0	6.5	3.0	10.0	7.5	6.5
14	13.0	9.0	3.0	9.0	9.0	3.0
21	11.0	6.5	4.0	11.0	5.5	4.0
28	9.0	4.0	2.0	10.0	4.0	4.0
5 December	7.5	2.0	2.0	7.5	5.5	2.0
12	7.5	6.5	2.0	6.5	5.5	2.0
19	9.0	5.5	3.0	7.5	4.0	2.0
26	7.5	7.5	2.0	7.5	9.0	3.0
2 January	9.0	3.0	2.0	9.0	7.5	3.0

The values in Table 31 d comparing lesion position and method of planting indicate that many primaries developed from neck infected bulbs planted in ridges, but nonetheless a sizeable proportion (44%) remained with infections restricted to the parent bulb.

In 1967/68 this experiment was repeated with slight alterations - sufficient bulbs were planted to allow recording to continue until senescence, with lesion position and not mode of planting being compared (Table 34 & Appendix 5). Temperatures were recorded throughout in a ridge (Fig. 9), and from spring onwards the soil moisture content was calculated weekly as soil tension in cms water (Appendix 1).

Fewer successful inoculations were recorded than in the previous year, possibly because of unfavourably dry storage conditions after inoculation. All the possible infection pathways occurred excepting the appearance of primaries. Whereas B. tulipae was restricted to c. 70% of stock bulbs throughout 1966/67, it continued to spread to underground sections of stem as the season advanced - the numbers of stems infected increasing from 3 during the planting to emergence period to 68 from heading to senescence. Why were there so few primaries? The critical period for foliar infection in this season was December and January. The rate of shoot emergence, which was similar to the previous season, was approximately two weeks more advanced. Growth by the host seemed to be independent of the recorded temperatures, these may have differentially affected the fungus, lower temperatures having an adverse effect and minimising shoot infection (Fig. 7).

During the third period of recording, from heading to senescence, seven middle and six neck-infected bulb clusters

Table 34

EFFECTS OF DIFFERENT SITES OF INOCULATION ON THE DEVELOPMENT OF TULIP FIRE EXPRESSED AS PERCENTAGES 1967/68

		Distribution of infection		
	Shoot not emerging	Shoot emerging		
	Mother bulb rotted	Mother bulb rots		
		Shoot healthy	Shoot infected	
			Stem	Leaf
(a) Changes in expression of disease during the growing season				
Sampling period				
Planting - emergence	1	96	3	0
Emergence - heading	13	70	17	0
Heading - senescence	17	15	68	0
Seasonal value	8	72	20	0
(b) Mean effects of different sites of inoculation				
Middle of bulb	3	77	19	1
Neck of bulb	12	22	66	0

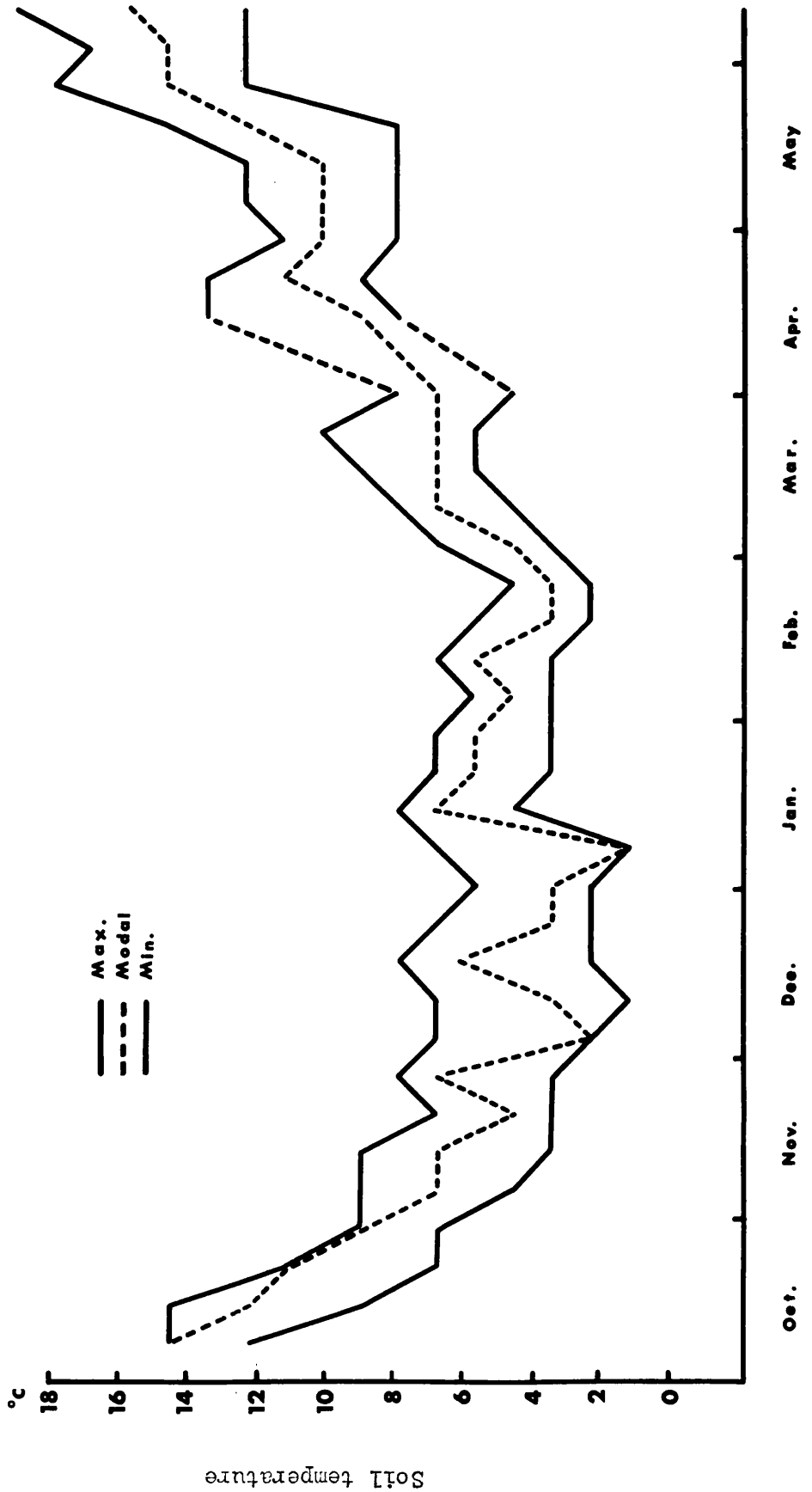


Fig. 9. Soil temperatures recorded at bulb depth in ridges, 1967/68.

contained infected daughter bulbs as a result of B. tulipae following different infection pathways.

At senescence in early July, 90 bulb clusters from each treatment were lifted and examined; of these twelve middle and ten neck-infected bulb clusters contained diseased daughter bulbs. Apart from confirming the presence of underground infection pathways that are symptomless above-ground, the absence of primaries, which might lead to secondary leaf spotting and subsequent daughter bulb infection, showed the efficiency of the below-ground pathway in the perennation of the disease.

Once the underground pathway had been found it was clear that other factors might interact with lesion position to influence the expression of fire. For example, large bulbs, the grade usually retained by dry bulb producers for stock, when infected might be more likely to develop the underground disease pattern because of the longer distance involved, whereas small bulbs might rot completely.

Time of planting could conceivably influence disease expression, especially if low soil temperatures affect the fungus and host differentially. The difference in disease development already found in ridge and bed planted tulips might be an indication that depth of planting also exerts an influence on the disease.

(b) (2) Effects of temperature on disease development. Shoot growth out-of-doors during autumn and winter of 1966/67 and 1967/68 was linear despite fluctuating soil temperatures, which tended to decrease in early winter (Fig. 5). This lack of major changes in growth rate suggests that growth was, to some extent, independent of temperature, contrasting strongly with temperature effects on

B. tulipae cultures in vitro (Fig. 1). To discover the effects of temperature on the host-pathogen complex, necks of tulip bulbs were inoculated with sclerotia and planted in boxes which were placed in soil tanks maintained at 4°, 10° and 15.5°C. At intervals, bulbs were lifted, shoot lengths measured and lesion growth assessed. The rate of shoot elongation was considerably less at 4°C than at 10 and 15.5°C, where rates were similar (Table 35 & Fig. 10).

Table 35

SHOOT EXTENSION AT DIFFERENT SOIL TEMPERATURES OF BULBS INOCULATED WITH B. tulipae

Number of days after planting	Growing temperature °C		
	4°	10°	15.5°
	Shoot extension (mm)*		
41	14.6	25.0	21.4
56	20.3	42.5	29.4
70	29.6	66.9	79.5
82	51.0	153.1	109.2

* Cumulative totals

The inoculated bulbs on examination were placed into one of three categories on a scale of increasing disease severity:

(1) No apparent spread of fungus from site of infection,

(2) Bulb rotting but shoot healthy and,

(3) Bulb rotting with diseased shoot.

The results (Table 36) show that higher temperatures favoured disease development. Within 41 days of planting B. tulipae had

Fig. 10. Effects of differing soil temperatures on shoot elongation of tulips.

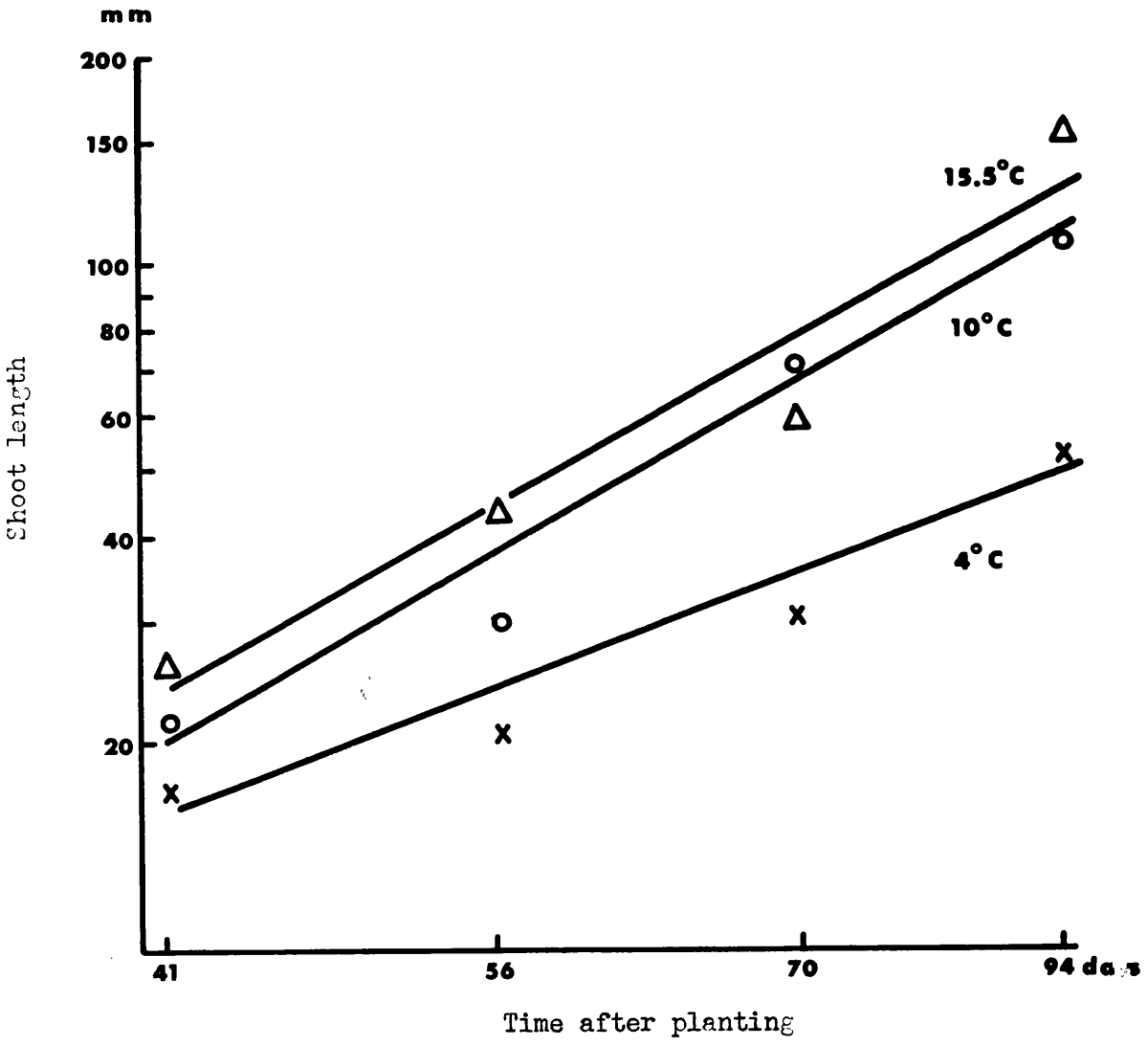


Table 36

THE CHANGING PATTERN OF ATTACK WHEN BULBS INOCULATED WITH *B. tulipae* WERE GROWN CONTINUOUSLY AT DIFFERENT SOIL TEMPERATURES

Soil temp.	No. of days after planting	Symptoms of attack: No apparent spread of pathogen from inoculation site	Mother bulb rotted: Shoot diseased	Mother bulb rotted: Shoot healthy
4°C	41	11	1	2
	56	1	4	9
	70	0	2	6
	82	0	1	6
	TOTAL	12	8	23
	% of total	28	19	53
10°C	41	2	9	3
	56	0	14	0
	70	0	4	4
	82	0	2	6
	TOTAL	2	29	13
	% of total	4	66	30
15.5°C	41	1	8	5
	56	6	7	1
	70	0	2	6
	82	0	3	5
	TOTAL	7	20	17
	% of total	16	45	39

Plate 14.

Plate 15.

caused appreciable rot on most bulbs at 10 and 15.5°C, but only three of fourteen inoculations were associated with spreading lesions at 4°C (Plate 14). This early attack was associated with the subsequent development of diseased shoots. Thus, the combined totals at 10 and 15.5°C indicate that 49 of 79 (62%) rotting bulbs developed diseased shoots but only 8 of 31 (26%) did so at 4°C.

To extend our knowledge of temperature effects, eight bulbs grown for 41 days at 4°C were examined and replanted at 15.5°C. Fourteen days later these and eight other bulbs, which had remained at 4°C were examined (Table 37 a). The higher temperature increased shoot elongation tenfold, mean growth increments being 37.1 mm (52.7 - 15.6) at 15.5°C and 3.5 mm (19.2 - 15.6) at 4°C, whereas measurements given in Table 1 indicate that the mean daily growth increments in vitro of the isolate of B. tulipae used in this experiment were four times greater at 15.5°C (22.7 mm) than at 5°C (5.6 mm). The growth rates of bulbs lifted 70 days after planting compared with mean daily growth increments of B. tulipae in culture, shows clearly that whereas there is little temperature effect on shoot elongation between 10 and 15.5°C, temperature continues to have a major effect on the growth of the fungus (Fig. 11).

These patterns of temperature response were reflected in a greater proportion of diseased shoots among bulbs moved from 4°C to 15.5°C (Table 37 b & Plate 15). Lesion size was also assessed according to whether it:

(1) remained as a small circular lesion,

(2) enlarged to reach the bulb tip, or

(3) encircled the neck, i.e. increasing severity of attack.

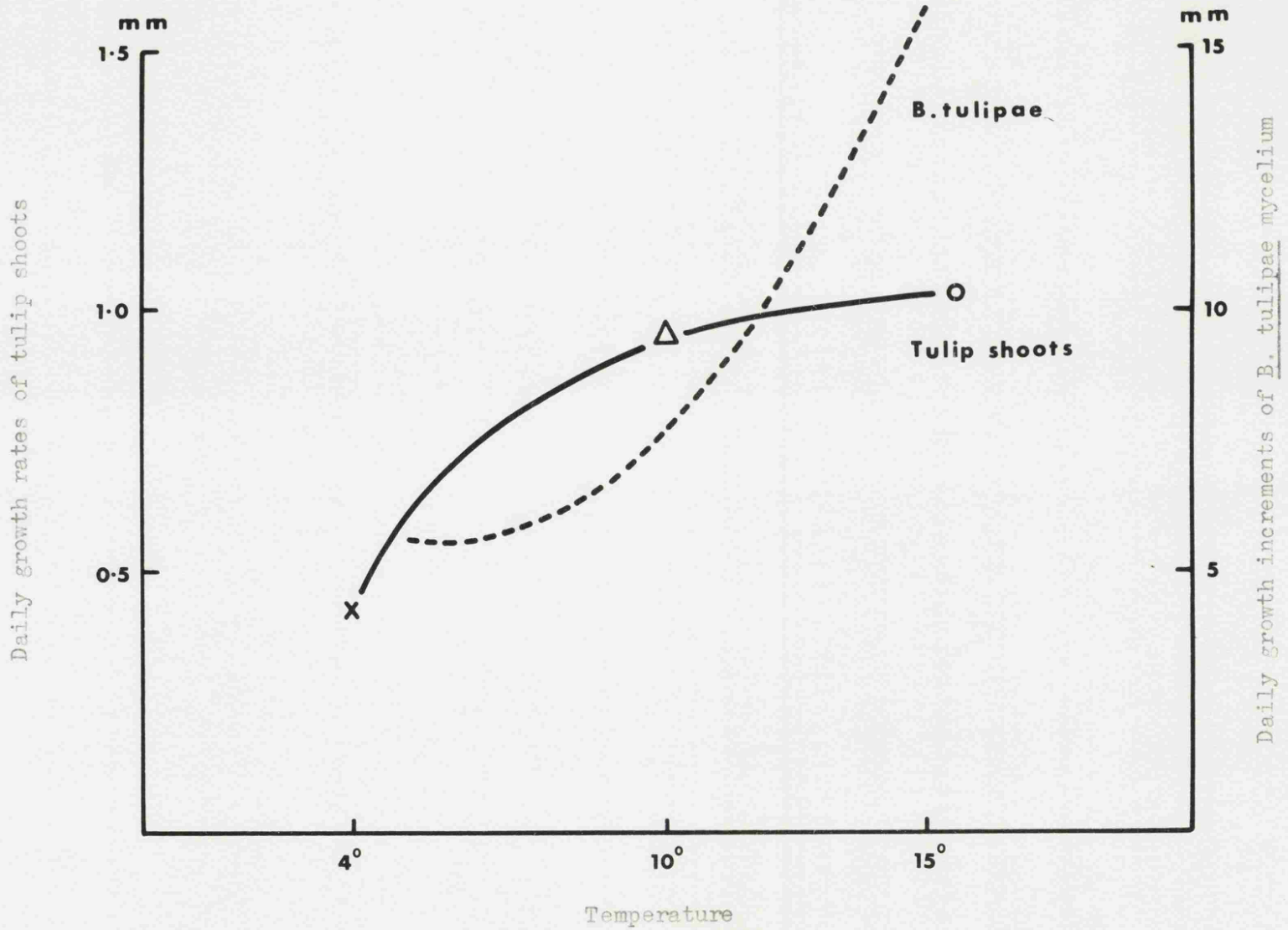
These results (Table 37 c) suggest that at lower soil temperatures

Table 37

EFFECT OF TEMPERATURE CHANGE ON THE DEVELOPMENT OF OXFORD BULBS INOCULATED WITH *B. tulipae*

	Conditions of incubation		
	41 days at 4°C	41 days at 4°C, 14 at 15.5°C	56 days at 4°C
(a) Shoot length (mm)			
Bulb 1	14	53	13
2	33	46	15
3	8	34	7
4	9	58	25
5	12	53	17
6	15	63	14
7	18	69	34
8	16	46	29
Mean	15.6	52.7	19.2
(b) Spread of *B. tulipae*			
1. No apparent spread from site of inoculation	7	0	1
2. Mother bulb rotting			
a. Shoot healthy	1	5	6
b. Shoot diseased	0	3	1
(c) Type of lesion			
a. Little or no extension	7	0	1
b. Lesion extending to bulb tip	0	0	3
c. Lesion encircling bulb	1	8	4

Fig. 11. Comparison between rates of tulip shoot elongation grown at 3 soil temperatures based upon 70 days growth and the growth rates of B. tulipae on malt agar at 3 similar temperatures.



lesion development is slow, but is enhanced if temperature is increased.

In general terms, low soil temperatures retard pathogen development sufficiently to allow shoots to escape infection. The percentage infection of mother bulbs is not reduced at this low soil temperature, but the expression of the disease is altered so that a greater proportion of bulbs is available for below-ground (symptomless) phases of disease. Warmer soil temperatures allow rapid disease development, which, in turn means that the chances of shoot infection, and thus the development of above-ground phases of disease are greater.

These observations give the detailed background to statements made by discerning growers that severe winters reduce the amount of fire.

(b) (3) Effect of bulb grade on disease development. The underground disease pathways are of two distinct types:

(1) the outer, when the pathogen moves down the outer fleshy scale and,

(2) the inner, when, shortly after the cessation of stem elongation, the pathogen moves along stems as well as fleshy scales.

The pathways followed by the pathogen will depend upon many factors, but probably lesion position and size of bulb are among the most important. In the bulb industry yield is rarely quoted in terms of weight, greater emphasis being placed upon size, sorting being done mechanically according to circumference. Thus, bulbs are sold according to grade.

To test effects of grade, twenty Elmus bulbs of each of five grades were inoculated in August 1967 in the neck region and, after storage, planted in late September in ridges (Appendix 6). By the following June, daughter bulbs had developed sufficiently to form small clusters but with sufficient mother bulb tissue remaining to enable the pathway taken by the pathogen to be determined. Unfortunately, there were few replicate bulbs but the results suggest that lesions are less likely to develop in large bulbs than in small ones (Table 38).

Table 38

EFFECT OF SIZE OF BULB (GRADE) ON DEVELOPMENT OF DISEASE IN ELMUS BULBS INOCULATED WITH B. tulipae

	Symptom of attack				
	Shoot emerging				Shoot not emerging, mother bulb rotted
		Mother bulb rotted			
Grade of bulb	No apparent spread of pathogen from inoculation site	Shoot healthy	Shoot infected		
			Stem	Leaf	
13-14 cm	6	9	5	0	0
11-12 cm	3	12	3	1	1
9-10 cm	4	12	3	1	0
7-8 cm*	3	7	7	0	3
6-7 cm*	1	11	2	0	6

* Including maidens

If the data for daughter bulbs produced from rotted bulbs, irrespective of stem infections above the neck are examined and separated according to infection pathway, differences in the efficiency of fungal transfer become clear (Table 39). Thirty four of forty nine (67%) were infected when B. tulipae followed the outer pathway, compared with fifteen of forty nine (31%) following the inner pathway; but in contrast, fewer of the individuals within clusters were infected by the outer pathway, i.e. twelve and forty nine per cent respectively. Furthermore, the type of pathway was reflected by the position of infected daughter bulbs. In thirteen of fifteen instances the first daughter bulbs were infected by B. tulipae following the inner pathway but only three of thirty four in the outer. Why should the transfer to daughter bulbs along one pathway be more efficient than the other? As the season progresses, healthy mother bulbs lose weight and are destroyed by saprophytes. If the inner pathway is considered to be a deep infection, the amount of diseased tissue may act as a barrier against the usual invading saprophytes and the daughter bulbs in a cluster remain to be infected by B. tulipae. In the outer (shallower) pathway, where intense competition from soil organisms persists, daughter bulbs may be exposed to relatively smaller amounts of inoculum.

When a maiden bulb commences growth in autumn a single leaf emerges through the neck and, for pathological purposes, is analogous to the floral shoot of larger bulbs. By spring, when the leaf is fully developed, the leaf in the neck region is hollow and within this cavity the daughter bulb develops. Occasionally one of the axillary buds will also develop so that by the end of the season, two bulbs may have been formed. Because of this different

Table 39

EFFECT OF GRADE OF MOTHER BULBS INOCULATED WITH B. tulipae ON SUBSEQUENT INFECTION PATHWAYS AND POSITION OF INFECTED DAUGHTER BULBS

Grade	Nos. diseased clusters	Total nos. bulbs within clusters		Position of diseased daughter bulbs				
		Healthy	Diseased	1st	2nd	3rd	4th	5th
(1) Outer pathway								
13-14 cm	9	34	2	0	0	0	2	-
11-12 cm	11	30	4	1	0	1	1	1
9-10 cm	11	20	4	1	3	0	-	-
7-8 cm*	3	3	2	1	1	-	-	-
6-7 cm*	-	-	-	-	-	-	-	-
TOTAL	34	87	12	3	4	1	3	1
% of total			12					
(2) Inner pathway								
13-14 cm	5	15	5	5	0	0	0	-
11-12 cm	3	6	5	2	2	1	-	-
9-10 cm	3	3	5	2	2	1	-	-
7-8 cm*	4	0	8	4	3	1	-	-
6-7 cm*	-	-	-	-	-	-	-	-
TOTAL	15	24	23	13	7	3	0	-
% of total			49					

*excluding maidens

mode of development from flowering bulbs patterns of disease in maiden bulbs have been kept separate. In the grade experiment, thirty three of the two smallest grades developed as maidens and of these twenty nine developed fire (Table 40 a). Thirty one per cent (9) rotted completely, in seventeen per cent (5) the attack involved mother bulb and leaf base and in twenty five per cent (15) only mother bulbs rotted. As with larger grades, the more extensive rots involving leaf bases gave rise to a greater proportion of infected daughter bulbs than those confined to the bulb i.e. 4 of 6 and 2 of 22 infected respectively (Table 40 b).

(b) (4) Effect of planting date on fire. Although further investigation is required to determine what causes bulb lesions to recommence growth after planting in autumn, one influential factor might be date of planting. To examine this possibility, the necks of 10 cm Elmus bulbs were inoculated simultaneously, irrespective of planting date, placed in 'Netlon' and planted in ridges at the end of August, September, October and early December (Appendix 7). Primaries were recorded on appearance in spring 1968. The bulbs were lifted in late May when infection pathways were still discernible and daughter bulb infection was taking place. The results of this experiment (Table 41) suggest that the later the planting the more destructive the pathogen. Thus, whereas the fungus was confined to 7 of 20 bulbs planted in August, it had spread from them all when planted in December. Although lesions did not spread appreciably in store, late planting, contrary to expectation, favoured the destruction of mother bulbs. This suggests that there are some internal biological or physiological changes within the host.

Table 40 a

NUMBERS AND PATTERN OF DISEASE DEVELOPMENT IN MAIDEN ELMUS BULBS OF TWO GRADES INOCULATED WITH B. tulipae

Grade	No apparent spread of pathogen from inoculation site	Symptom of attack: Bulb only rotted, leaf base healthy	Symptom of attack: Bulb and leaf base rotted	Symptom of attack: Complete rot	Total
7-8 cm	3	4	3	3	13
6-7 cm	1	11	2	6	20
TOTAL	4	15	5	9	33
% of diseased bulbs		52	17	31	

Table 40 b

EFFECTS OF INFECTION PATHWAYS ON NUMBERS OF INFECTED DAUGHTER BULBS PRODUCED BY INOCULATED MAIDEN ELMUS BULBS OF TWO GRADES

Infection pathway	Grade	Daughter bulbs examined: Total numbers	Daughter bulbs examined: Numbers infected
Bulb	7-8 cm	4	1
	6-7 cm	18	1
Bulb and leaf base	7-8 cm	3	2
	6-7 cm	3	2
	TOTAL	28	6

Table 41

EFFECT OF PLANTING DATE ON INFECTION PATHWAYS OF BULBS INOCULATED WITH *B. tulipae* IN EARLY AUGUST

	Symptom of attack				
	Shoot emerging				Shoot not emerging, mother bulb rotted
Planting date	No apparent spread of pathogen from inoculation site	Mother bulb rotted			
		Shoot healthy	Shoot infected		
			Stem	Leaf	
August *	7	1	7	4	1
September	4	6	9	1	0
October	1	1	3	1	4
December	0	2	9	3	6 & 4 †

*20 bulbs/sample, except in October which was 10 bulbs

† 2 foliar and 2 stem primaries that subsequently rotted completely

Numbers of clusters containing healthy and infected daughter bulbs are given in table 42 a, and it appears that the increased activity of *B. tulipae* in later planted bulbs leads to an increased rate of daughter bulb infection. In Table 42 b numbers of infected daughter bulb clusters have been rearranged according to infection pathways described and as before the pathogen transferred more readily via the inner pathway; 74% of the clusters containing infected bulbs compared with 36% via the outer pathway.

(b) (5) Effects of planting depth on fire. Comparison of disease patterns in ridge and bed planted bulbs which were neck-inoculated in 1966 (Appendix 3) suggested that infected bulbs planted in ridges were more likely to become primaries than those

Table 42 a

EFFECTS OF INFECTED AND HEALTHY BULB CLUSTERS RESULTING FROM ROTTING MOTHER BULBS WHICH DID NOT GIVE RISE TO PRIMARIES

Planting date	Clusters		Infected clusters as % of total
	Healthy	Infected	
August	4	4	50
September	7	8	53
October	1	4	80
December	2	8	80

Table 42 b

NUMBERS OF INFECTED AND HEALTHY BULB CLUSTERS RESULTING FROM INFECTED MOTHER BULBS VIA DIFFERENT INFECTION PATHWAYS

Planting date	Inner pathway		Outer pathway	
	Healthy	Diseased	Healthy	Diseased
August	4	3	0	1
September	3	6	4	2
October	0	4	1	0
December	0	7	7	4
TOTAL	27		11	
% of infected bulbs	74		36	

in beds which were attacked symptomlessly below ground. To check this result, neck-inoculated 10 cm bulbs of two cultivars, Rose Copland and William Pitt, were planted at four depths (Appendix 8). The experiment was duplicated to allow two liftings, an earlier one in May to record infection pathways below ground (Table 43) and one at senescence in June to record daughter bulb infection (Table 44). The results suggest that planting depth did not influence the development of tulip fire, which, however, differed on the two cultivars tested. In the first sample lifted in May (Table 45) numbers of bulbs with above or below ground symptoms of fire were analysed using the χ^2 test. In other words, are differences in symptom expression between the two varieties simply because of the size of sample or is there a fundamental difference? This statistical test enables the magnitude of the difference between observed and expected values to be compared and determines the possibility of these differences exceeding the calculated value for χ^2. Although numbers of rotted mother bulbs did not differ especially, the differing numbers of healthy and diseased shoots of William Pitt suggest that bulb infections are more likely to be translated to primaries than those of Rose Copland.

The results of the final lifting at senescence in June show a similar trend to that observed in the first (Table 44). An analysis of numbers of infected daughter bulb clusters formed at the different depths show no statistically significant differences but total numbers of infected clusters of the two varieties again indicate that William Pitt is more readily damaged than Rose Copland (Table 46).

Table 43

EFFECT OF PLANTING DEPTH ON INFECTION IN 10 CM BULBS OF ROSE COPLAND AND WILLIAM PITT, INOCULATED AND PLANTED IN AUTUMN 1967 AND LIFTED IN MAY 1968

Planting Depth	Cultivar	Symptom of attack: Shoot emerging — No apparent spread of pathogen from inoculation site	Shoot emerging — Mother bulb rotted — Shoot healthy	Shoot emerging — Mother bulb rotted — Shoot infected — Stem	Shoot emerging — Mother bulb rotted — Shoot infected — Leaf
Ridge	Copland	19	17	3	1
	Pitt	15	10	15	0
10 cm	Copland	5	29	6	0
	Pitt	10	21	9	0
20 cm	Copland	1	35	4	0
	Pitt	10	20	9	1
30 cm	Copland	6	23	10	1
	Pitt	11	13	15	1

Table 45

COMPARISON OF DISEASE SYMPTOMS OCCURRING IN INOCULATED BULBS OF WILLIAM PITT AND ROSE COPLAND IN AUTUMN 1967 AND LIFTED IN MAY 1968

Cultivar		Total nos. of bulbs rotting	Nos. bulbs associated with mother bulb rotting	
			Diseased Shoots	Healthy Shoots
William Pitt	Observed	115	50	64
	Expected		35.19	78.81
Rose Copland	Observed	129	25	104
	Expected		39.81	89.19
Total	Observed	243	75	168

χ^2 value for total numbers = not significant

χ^2 value for diseased shoots = 11.745

χ^2 value for healthy shoots = 5.511

The bulb clusters formed at the different depths by the two varieties were separated and numbers of diseased bulbs recorded. Using the χ^2 test (Table 47) the proportions of William Pitt bulbs diseased at the different depths were not statistically significant. In contrast, disease incidence was greatest on Rose Copland bulbs formed at 30 cm depth of planting but tthis result needs further confirmation.

Table 46

EFFECT OF CULTIVAR ON BELOW GROUND INFECTION OF BULB CLUSTERS FORMED FROM INOCULATED MOTHER BULBS PLANTED AT DIFFERENT DEPTHS, LIFTED IN JUNE 1968

Cultivar	No. of clusters infected			Infected as % of total
	Observed	Expected	Total	
William Pitt	145	122.5	160	91
Rose Copland	100	122.5	160	64
TOTAL	245	245.0	320	77

$\chi^2 = 7.902$ (P 0.01 = 6.635)

Table 47

EFFECT OF PLANTING DEPTH ON THE BELOW GROUND INFECTION OF DAUGHTER BULBS OF ROSE COPLAND AND WILLIAM PITT LIFTED IN JUNE 1968

Cultivar	Planting depth	No. infected bulbs Observed	Expected	Total no. of bulbs	Infected as % of total
	Ridge	85	78.42	121	70.2
William	10 cm	57	62.87	97	58.8
Pitt	20 cm	62	58.33	90	68.9
	30 cm	52	56.38	87	59.8
	TOTAL	256	256.00	395	

χ^2 = 1.6714 not significant

	Ridge	45	46.92	136	33.1
Rose	10 cm	34	47.27	137	24.8
Copland	20 cm	41	48.65	141	29.1
	30 cm	67	44.16	128	52.3
	TOTAL	187	187.00	542	

χ^2 = 16.820 (P 0.001 = 16.266)

(b) Comparison between cultivars at all depths of planting.

William Pitt	256	186.75	395	64.8
Rose Copland	187	256.25	542	34.5
TOTAL	443	443.00	937	

χ^2 = 38.315 (P 0.001 = 16.266)

Plates 16 & 19.

B.tulipae sclerotia

Plate 16. On fleshy scale lesion.

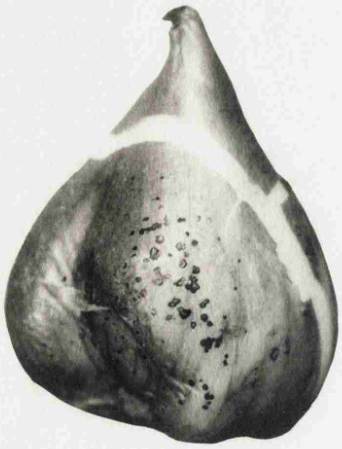


Plate 17. On bulb tunic.

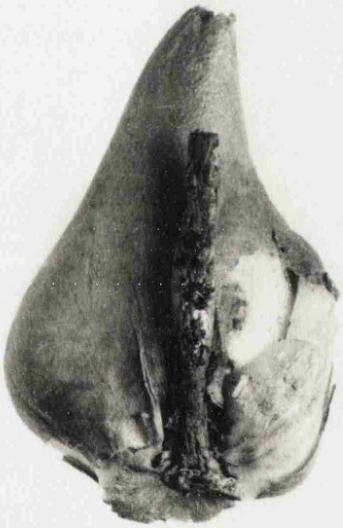


Plate 18. On previous seasons flower stalk.

Plate 19. On Tulipa fosteriana seeds.

(b) (6) Sclerotia. In the past many assumptions have been made about the importance of sclerotia but the fact that mycelium of B. tulipae can perennate successfully on bulbs does not mean that sclerotia are unimportant. The latter have been found on many parts of the tulip, including fleshy scales (Plate 16), the tunic (Plate 17), the remains of previous season's flower stalks (Plate 18) and seeds (Plate 19).

Hopkins (1921) stated that B. tulipae survived on bulbs during 'dormant' periods as mycelium or sclerotia in soil. Beaumont et al (1936) considered that sclerotia in soil were potentially more dangerous than the few found on bulbs, believing that a 2 year break in the rotation cycle to be insufficient to effect a significant inoculum decrease. Because symptomless underground pathways were unknown at that time, many symptoms of disease were attributed to invasion by soil-borne sclerotia. Observations (Table 28) of diseased tulips grown on land not previously planted with this crop suggest, however, that infection resulting from soil-borne sclerotia would, in most instances, be masked by a greater incidence attributable to fungal propagules perennating on bulbs.

To test the efficacy of soil inocula, sclerotia formed on malt agar petri dish cultures were dried and placed on or near healthy 10 cm Elmus bulbs planted singly in 14 cm pots containing J.I.1. potting compost. The five treatments, listed in Table 48, were replicated eight times; the pots, each containing sclerotia from one petri dish culture, being subsequently stood out-of-doors.

Shoots of bulbs, planted in October, emerged in early March and aerial symptoms were subsequently recorded at monthly intervals. Total numbers of primaries, abnormally small shoots

B.tulipae sclerotia

Plate 16. On fleshy scale lesion.

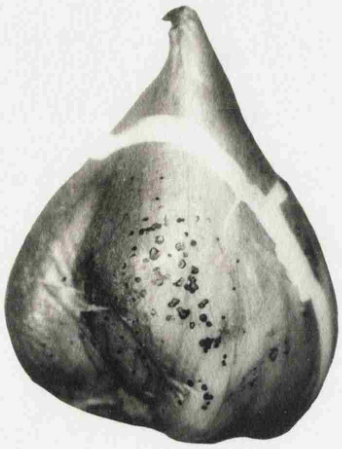
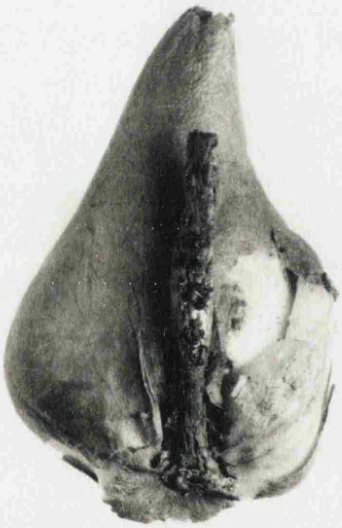


Plate 17. On bulb tunic.



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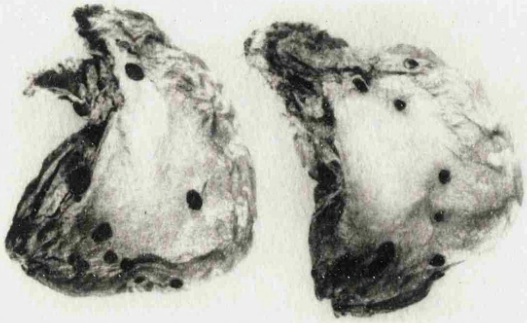

Plate 19. On Tulipa fosteriana seeds.

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Shoots of bulbs, planted in October, emerged in early March and aerial symptoms were subsequently recorded at monthly intervals. Total numbers of primaries, abnormally small shoots

and unemerged bulbs recorded in Table 48, suggest that sclerotia were damaging only when near to developing shoots. However, when observations of aerial structures were supplemented in July by an examination of bulbs, different patterns of attack were revealed (Table 49). Many plants with apparently healthy above-ground shoots were found to be attacked to a greater or lesser extent. When sclerotia were placed 38 mm below bulbs, only 4 of 8 bulbs were invaded (Treatment 4). Where sclerotia were above or attached to the bulb (Treatments 1 & 5) the entire crop was invaded with B. tulipae causing complete rot of nearly half. In the remaining treatments, fewer mother bulbs were diseased but nevertheless more daughter bulbs were colonised.

Table 48

EFFECTS OF INFESTING BULBS AND/OR SOIL WITH SCLEROTIA ON THE DEVELOPMENT OF TULIP FIRE MEASURED BY THE TOTAL NUMBER OF BULBS WITH NON-EMERGENT AND PRIMARILY INFECTED EMERGING SHOOTS

Site of bulb and/or soil infestation	Examination for presence or absence of infected shoots		
	March	April	May
	(Nos. of a total of 8 replicates)		
19 mm above bulb	5	6	7
Taped to flower stalk	4	5	7
Around bulb, but 19 mm distant	0 0	0	0
19 mm below bulb	0	0	0
38 mm below bulb	1	1	1

Table 49

EFFECTS OF INFESTING BULBS AND/OR SOIL WITH SCLEROTIA ON THE UNDERGROUND DEVELOPMENT OF *B. tulipae* ON MOTHER AND DEVELOPING DAUGHTER BULBS

Sites of bulb and/or soil infestation	End of season (June) incidence of *B. tulipae* on			
	Mother bulbs		Daughter bulbs	
	Nos. of		Nos. of clusters with infected daughter bulbs	Nos. of bulbs in infected clusters
	Infected	Healthy		
19 mm above bulb	7 (3)†	0	3	4/9*
Taped to bulb	8 (3)	0	3	10/13
Around bulb	6	2	5	10/32
19 mm below bulb	6	2	5	11/33
38 mm below bulb	4	4	3	9/33

* Numerator equals number of diseased daughter bulbs from surviving mothers; denominator equals the total number of daughter bulbs from surviving mother bulbs.

† Number in brackets equal bulbs totally rotted.

In this experiment, bulbs were planted in partially sterilised soil and as a result germinating sclerotia probably would not have competed against the full range of micro-organisms occurring in unsterilised soils. Nonetheless, because sclerotia 19 mm above bulbs caused damage sooner than those below bulbs, it seems that their activity is not overwhelmingly affected by root exudates.

Once again, the importance of the below ground phase of the disease is stressed because the incidence of shoot infection detectable in March, April and May was 31, 37 and 47% whereas 75% of bulbs were invaded when the incidence of B. tulipae was checked at lifting.

TULIP FIRE CONTROL

A. Introduction.

During the two decades before World War II most tulips were grown out-of-doors for cut flowers, with growers mainly concerned with loss of revenue attributable to flower spotting, decreased yields of dry bulbs being less important. In 1921, Hopkins recommen ed that the incidence of fire should be minimised by (1) planting pathogen-free bulbs and (2) removing primaries at an early stage in spring. Eleven years later Newton, Hastings and Bosher (1932) attempted to produce pathogen-free bulbs by dipping them in fungicidal solutions but with little success. Beaumont et al (1936) were similarly unsuccessful but their researches indicated that soil-borne, as well as bulb-borne, sclerotia could infect bulbs. Because of this observation it became uncommon for tulips to be grown on the same land at intervals of less than four years. Other unsuccessful attempts were made to reduce the incidence of foliar disease, at about this time, by increasing the depth of planting and/or delaying the time of planting (Holland County Council, 1932).

The acreage of bulbs was severely restricted during World War II. When production increased afterwards, a wider variety of fungicides was available together with more efficient spraying machinery, features that coincided with the formation of the National Agricultural Advisory Service (N.A.A.S.) which, in turn, initiated the development of Experimental Horticultural Stations. Two of these, at Rosewarne and at Kirton, are sited in the main centres of bulb production - namely Cornwall and Lincolnshire respectively.

Copper, carbamate, organic sulphur and salicylanilide formulations were compared in the early post-war spraying experiments when the value of carbamate compounds, especially zineb, soon became obvious if assessments of efficacy were based upon yield (Kirton E.H.S. 1960). Although bulbs after size grading are usually sold numerically, growers think in terms of weights, expressing the loss or increase during the season as a percentage of the weight planted.

Most experiments described in the literature relied on the natural incidence of B. tulipae, which was uncertain and rarely uniformly distributed. To increase experimental precision natural primaries were removed and one replanted in the centre of each plot, with assessments of leaf damage attributable to fire being made towards the end of the season. From the start of my thesis work in 1965 until 1967 the control of fire in the field was still orientated to procedures involving the regular roguing of primaries and the application of foliar sprays. Since the underground transmission of B. tulipae was recognised the scope of control measures has been greatly widened, more critical attention being paid to the control of B. tulipae when dormant on and/or in bulbs between lifting and planting.

B. Fungicidal spray experiments.

(a) Experimental design. By 1965 it was realised that rational spray programmes could be formulated only if the relation between leaf damage and yield loss was known, this in itself being dependant upon the optimal concentrations and frequencies of fungicidal applications. Experiments with similar planting densities but different plot layouts were started on widely separated sites at

Rosewarne and Kirton. To conform with local practice bulbs were planted in ridges at Rosewarne and in beds at Kirton (Fig. 12 a & b). To increase precision in determining the changing incidence of B. tulipae, successive observations were made weekly on the same marked plants. There were 8 marked plants per plot at Kirton and 7 at Rosewarne, being arranged systematically about the introduced primaries. After removing randomly distributed natural primaries an infector primary was planted in the centre of appropriate plots. Disease was assessed on an arbitrary scale -

No disease	0
Disease present	2
Up to 50% leaf area infected	4
50 - 70% leaf area infected	6
Very severe infection	8
Leaf senescing	10
Death	12

These observations integrated the incidence of aggressive and other lesions, the date of appearance of aggressive lesions being separately recorded.

(b) Natural spread of B. tulipae. The use of leaf assessments in plots not receiving fungicidal sprays but containing primary infectors enabled the pattern of disease development to be studied. The weekly mean assessment for tulip fire on all 8 plants in the unsprayed plots in 1966 and 1967 at Kirton are given in Figs. 13 & 14, the increments of disease differing considerably. The weekly fluctuations probably reflect changes in rates of sporulation and numbers of successful infections, which have been

Fig. 12a. Plot layout and arrangement of tagged plants used for successive disease recordings in fungicide spray experiments at Rosewarne E.H.S.

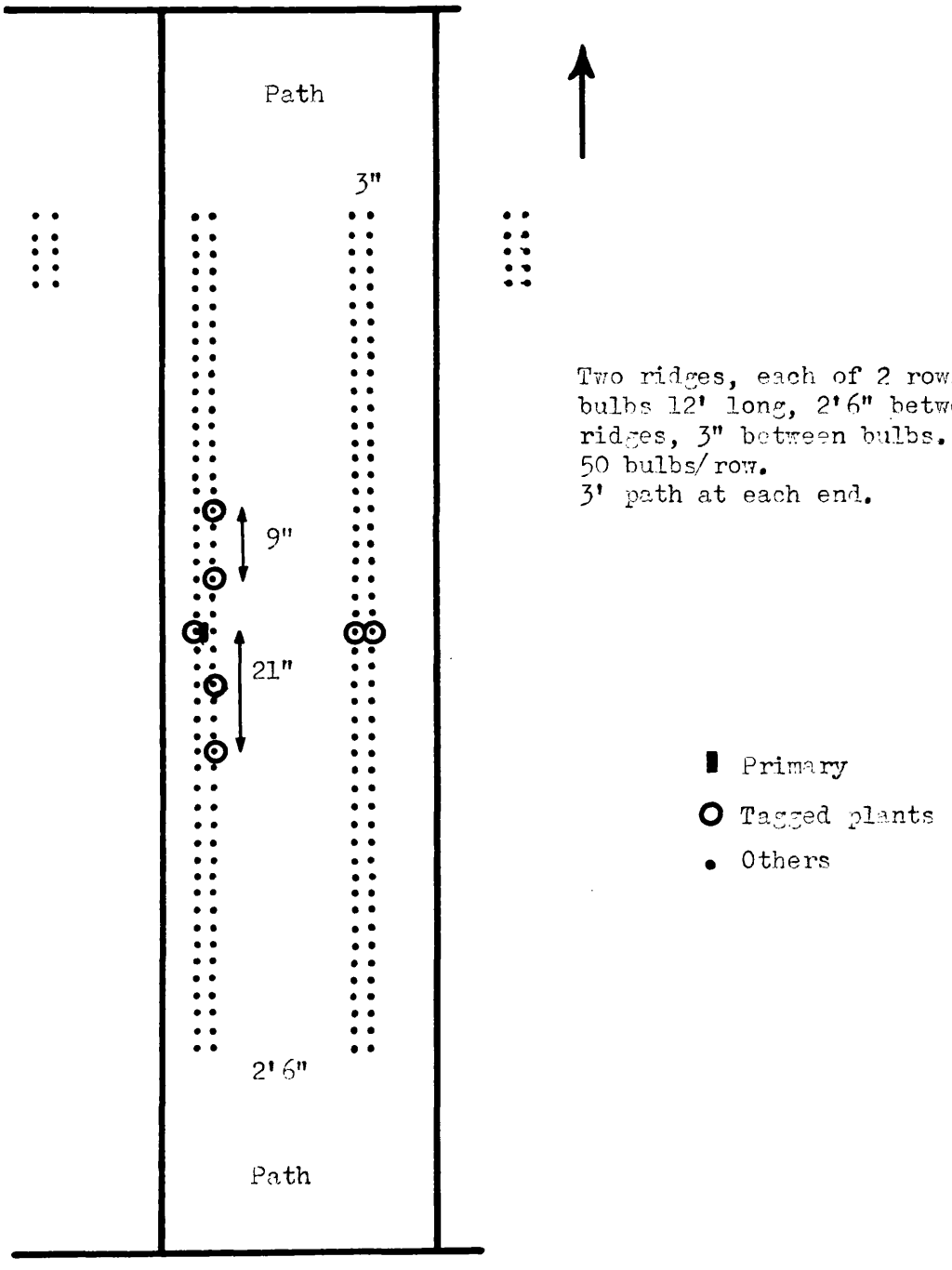


Fig. 12b. Plot layout and arrangement of tagged plants used for successive disease recordings in fungicide spray experiments at Kirton E.H.S.

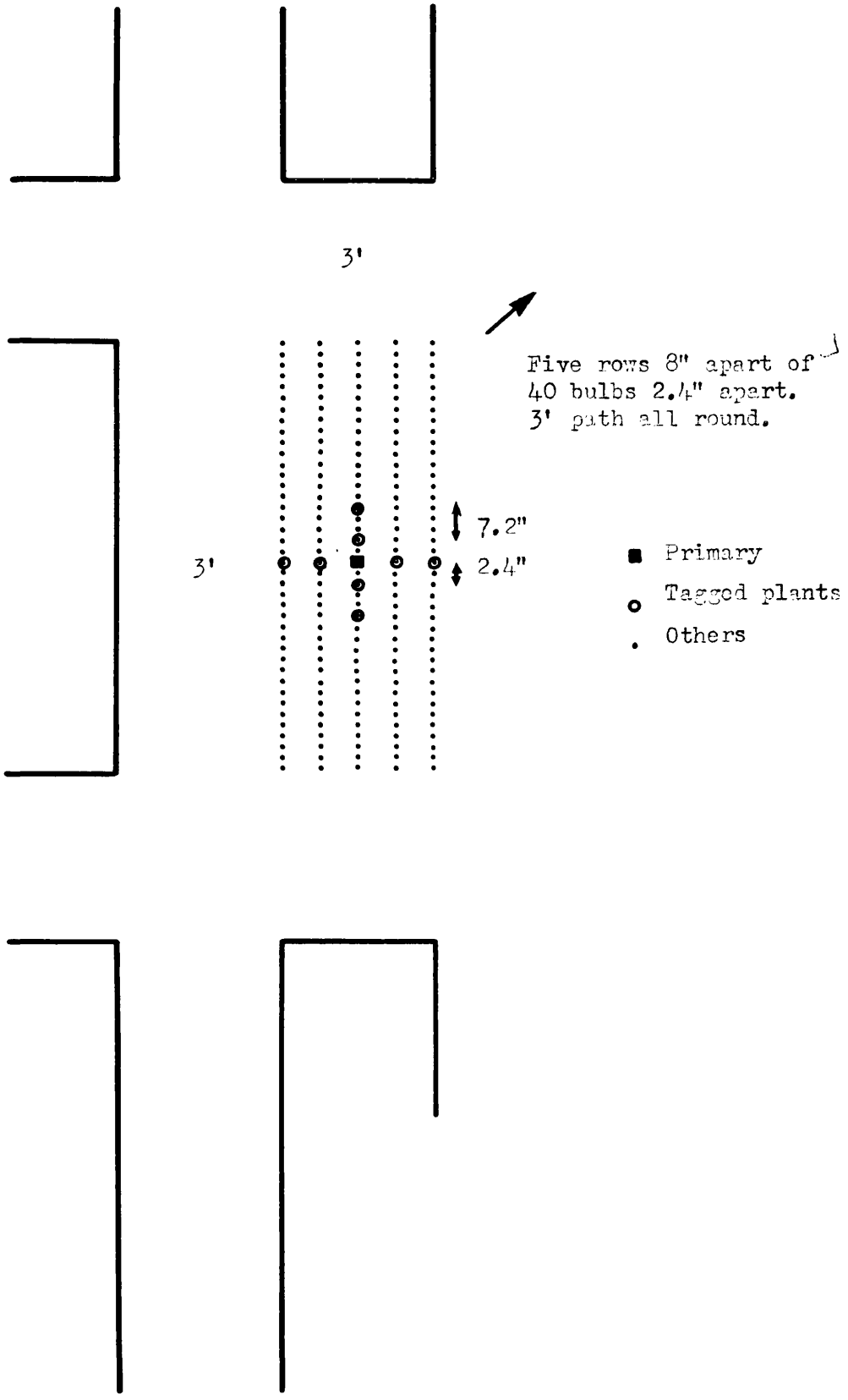


Fig. 13. Association of meteorological data and leaf disease integrals Kirton 1966

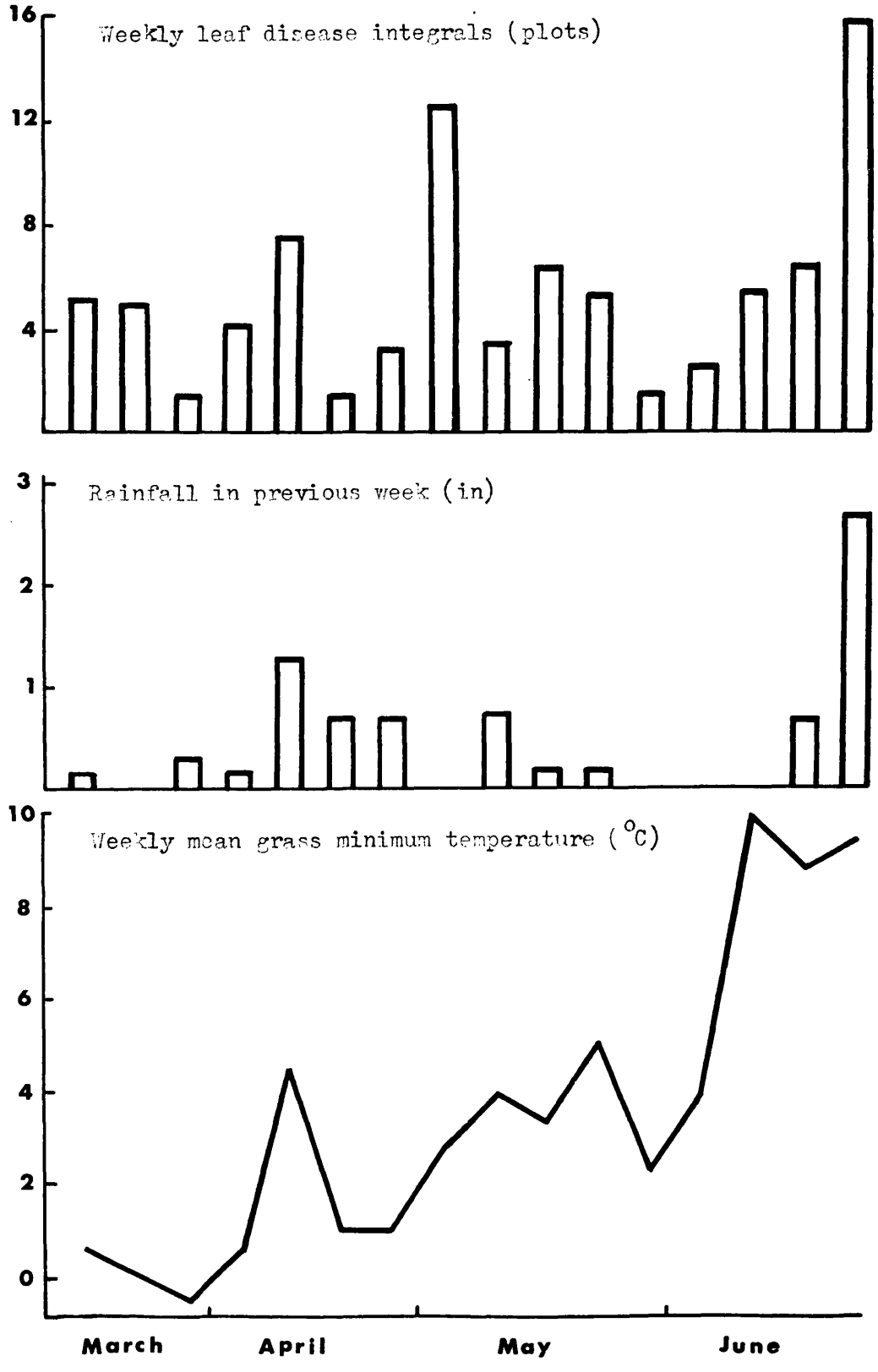
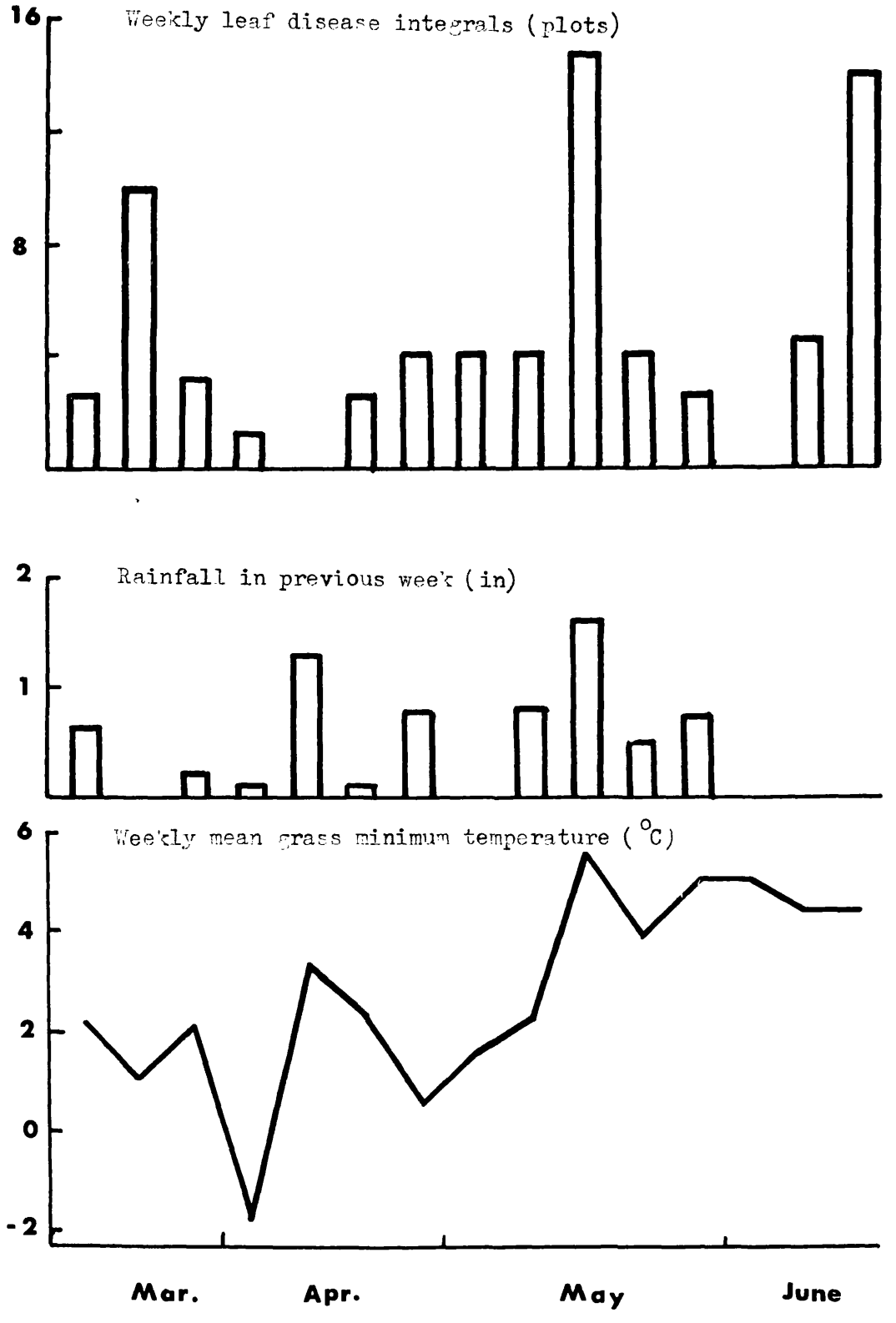


Fig. 14. Association of meteorological data and leaf disease integrals Kirton 1967



shown to depend on the persistence of water films and equitable temperatures. To associate these weather criteria with the changing incidence of B. tulipae, the weekly incidences have been plotted against the rainfall of the previous week and the weekly mean grass minimum temperature, but unfortunately records of dew were not available. Although there are insufficient data to test hypotheses mathematically, there seems to be some association between disease incidence and (a) the previous weeks rainfall and (b) the rising mean grass minimum temperature after a lag period; factors (a) and (b) influencing conidial dispersal, rates of penetration and lesion development. The cumulative weekly leaf disease integrals indicate that variation between the 6 unsprayed replicate plots at Kirton in 1966 was small with disease severity increasing as the season advanced, leaves senescing early (compared with sprayed plots) at the end of June.

It was not until the end of April, when accumulated disease integrals reached 50, that the first aggressive lesions were seen (Fig. 15). Until then, because non-aggressive lesions do not sporulate, the formation of lesions must be attributed to the primary sources of inocula, but once these lesions capable of sporulation were present, it is surprising that the rate of disease build-up did not subsequently accelerate. One explanation is that the method of leaf disease assessment based on the 2n sequence is faulty and instead, the eye is only capable of accurately perceiving changes on a n^2 scale. When the 2n series (2,4,6,8,10 & 12) was transformed to n^2 (2,4,8,16,32 & 64) the two lines diverged (Fig. 16) when aggressive lesions were first recorded. This suggested that the latter method, because it corresponds closely with expectation, may have more merit and will have to be checked by comparing actual leaf surface and lesion areas.

Fig. 15. Cumulative leaf disease integrals for 6 unsprayed plots exposed to B. tulipae inocula Kirton 1966

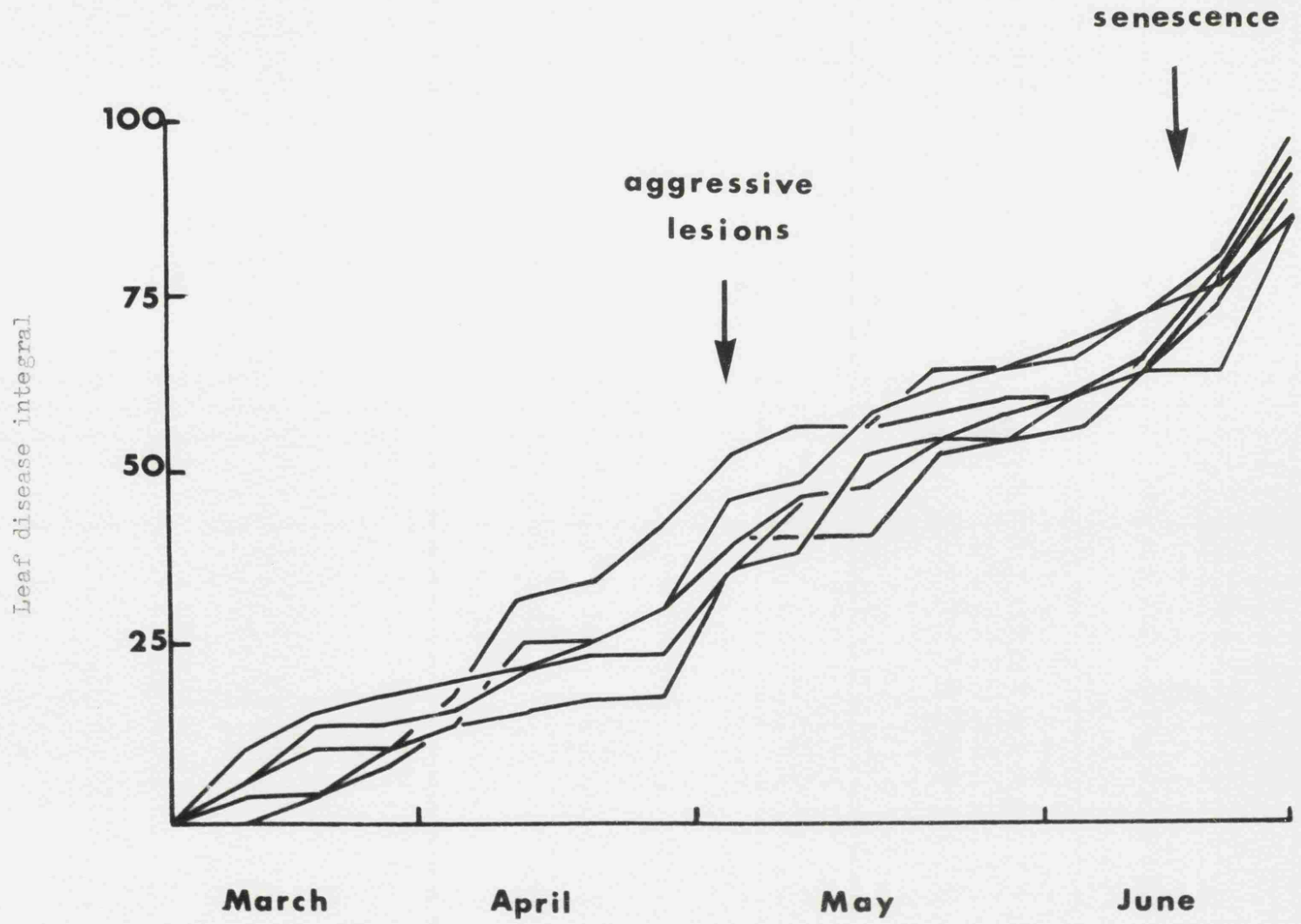
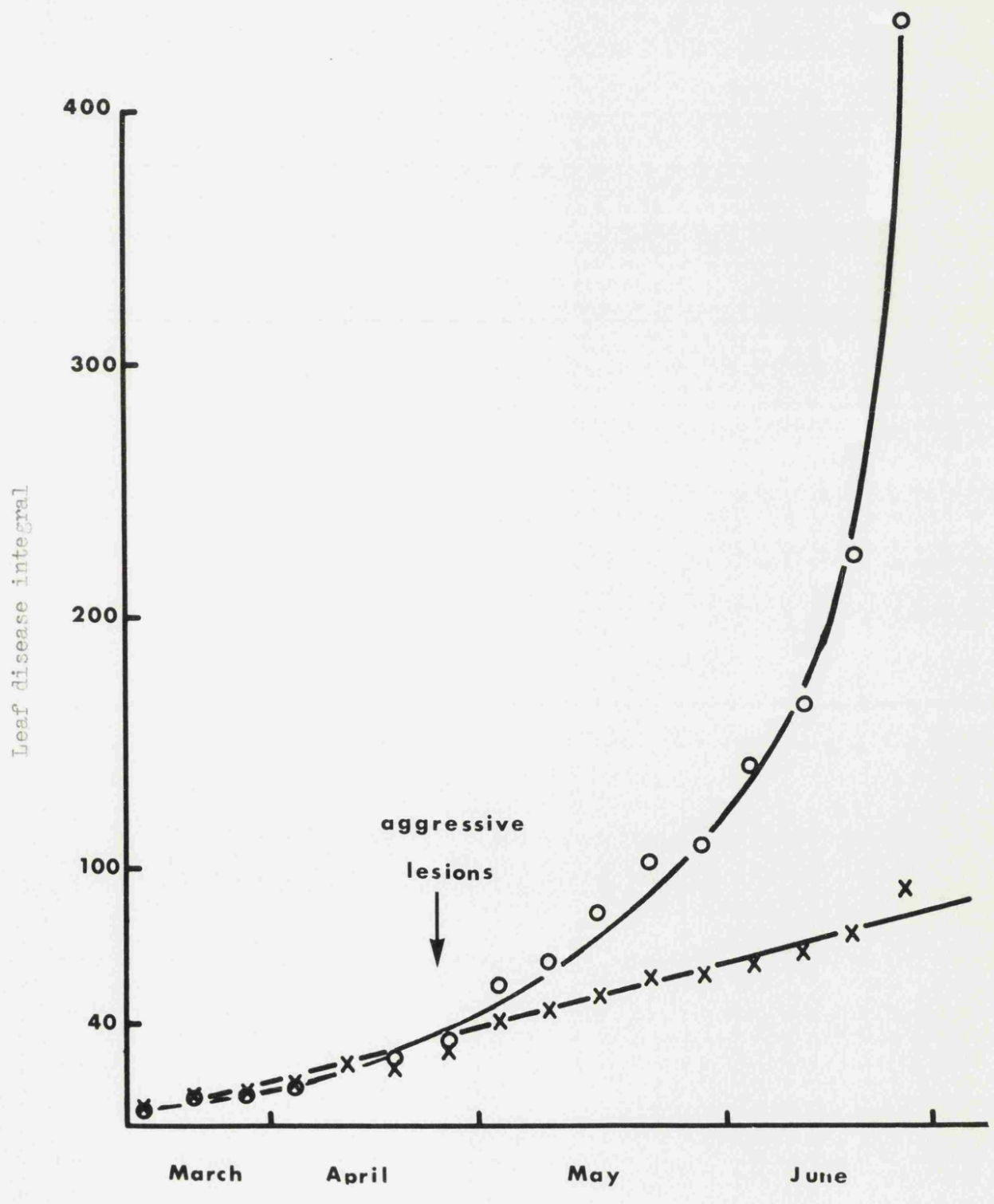


Fig. 16. Mean weekly cumulative leaf disease integrals for 6 unsprayed plots exposed to B. tulipae inocula at Kirton 1966. Two methods of assessment compared.



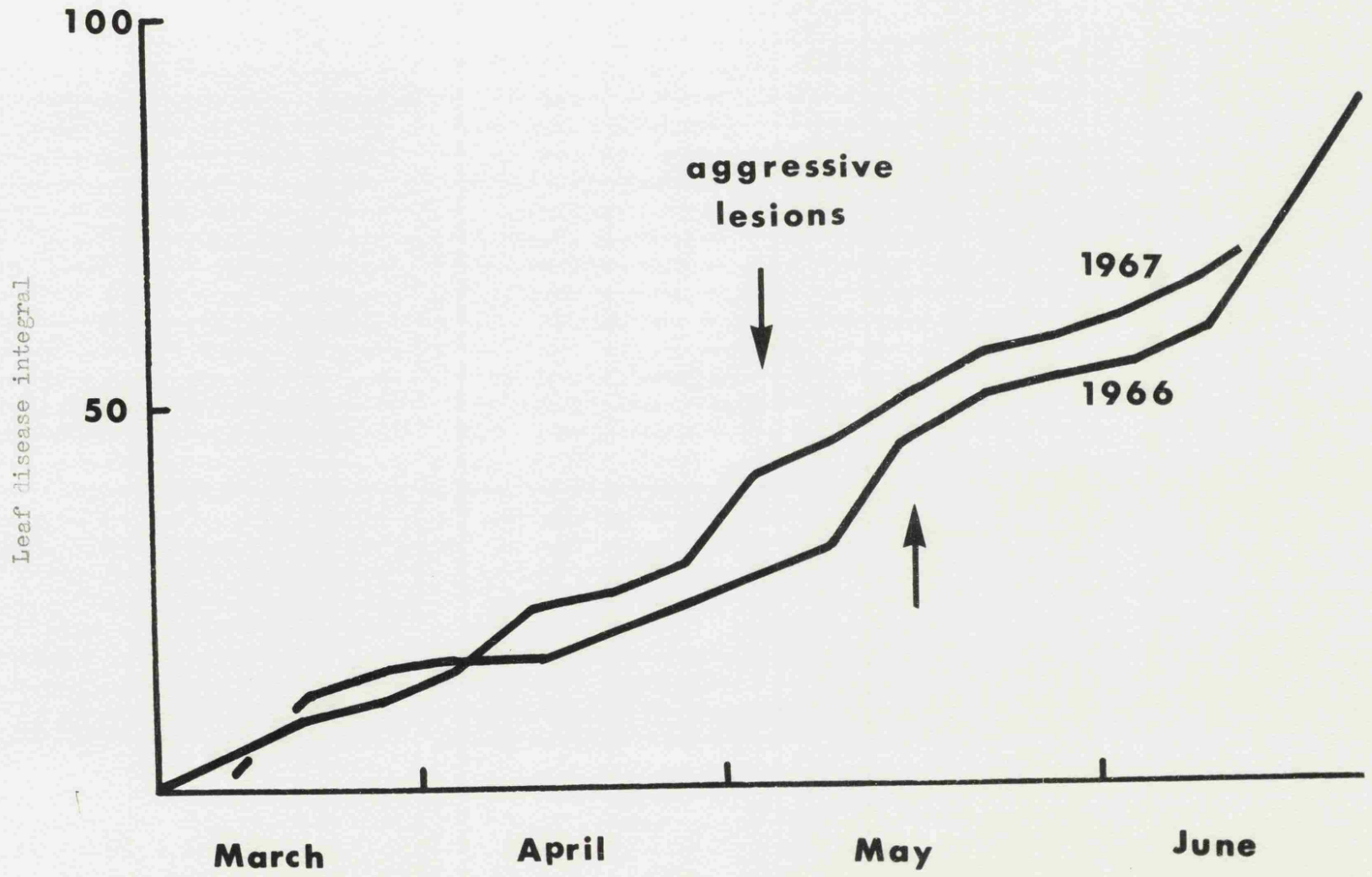
The progress of fire in different years may be compared, assuming that there are no major operator errors in disease assessment. The most noticeable feature of the curves for 1966 and 1967 at Kirton is the first appearance of aggressive lesions when the integral values lay between 40 and 50, a fortnight earlier in 1966 than 1967 (Fig. 17). This suggests that

(1) because non-aggressive lesions always appear before non-aggressive lesions there will always be a particular level of non-aggressive spotting before aggressive lesions are seen. This level being associated with the amounts of inocula to cause aggressive lesions.

(2) a particular combination of environmental factors occurs which permits lesion formation to develop into the aggressive phase. Although combinations of environmental factors favouring disease epidemics are well known (Beaumont and Mills Periods with Potato Blight and Apple Scab diseases) those for B. tulipae can only be deduced from laboratory experiments. These have shown the need for water films and large conidial concentrations for rapid lesion formation (Tables 19 & 21) and the influence of temperature on mycelial growth (Table 1). Or,

(3) host effects on lesion development, as Last (1955) showed with the incidence of Sporobolomyces on winter and spring sown cereals. Effects of differing dates of cereal planting were large but the two tulip experiments at Kirton were planted at similar times. The delay in aggressive lesion development cannot, therefore, be related to tome of planting effects. The experiment

Fig. 17. The incidence of B. tulipae on unsprayed tulips at Kirton E.H.S. during the 1966 and 1967 seasons



at Rosewarne were planted c. 2 weeks earlier than at Kirton and emergence was also 2 weeks earlier yet aggressive lesions were observed 6 weeks earlier (Fig. 18).

(c) Results of 1965/66 at Kirton and Rosewarne E.H.S.

Rosewarne. In 1965/66, the first year of this series of experiments, the aim was to assess the merits of the commonly used proprietary compounds, including captan and differing dithiocarbamates (Appendix 9).

When the weekly assessments of tulip fire incidence were summed, the totals integrating mean infection with time, it was found that fortnightly sprays (starting in early March) of Murphane Wettable, Murphane Wettable plus Orthocide, Dithane 945, Polyram tank-mix zineb and Fernide, significantly decreased infection (Table 50).

Table 50

EFFECT OF FUNGICIDAL SPRAYS ON (A) LEAF DISEASE INTEGRALS AND (B) YIELD INCREASES OF KRELAGE TRIUMPH BULBS AT ROSEWARNE 1965/66

Treatment	Disease integrals	Weight lifted (lbs)	% * increase
Dithane 945	193	18.8	27
Polyram	216	19.9	35
Tank-mix zineb	218	20.1	36
Fernide	245	19.0	28
Orthocide Murphane Wettable	250	20.6	40
Murphane Wettable	264	19.4	31
Unsprayed control	309	17.9	24

* calculated as increase over planting weight

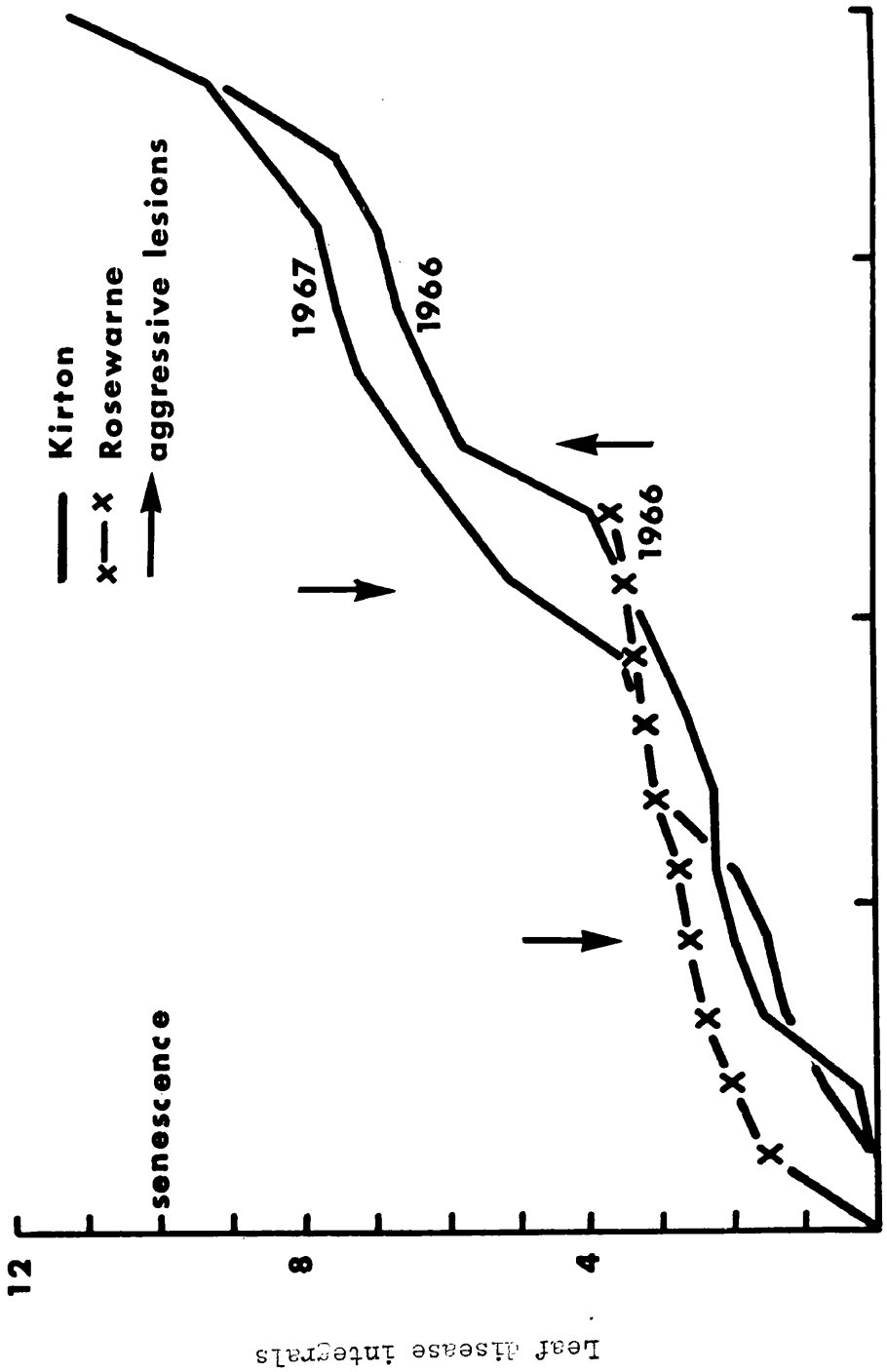


Fig. 18. Mean cumulative leaf disease integrals for plants in unsprayed plots at Kirton and Rosewarne E. H.S.

The yield increments were unexpectedly small - possibly attributable to less than usual amounts of sunshine in April causing, in turn, low net assimilation rates. Watson (1947) showed, using a range of arable crops, that dry matter increase varies with season and from year to year, the difference being principally associated with temperature. Rees (private com.) associated abnormally small bulb increases in Sussex during 1966 with lower than average amounts of solar radiation, which would be closely related to air temperatures.

Kirton. As at Rosewarne, the aim was to assess the merits of the commonly used proprietary compounds. Eight fungicides or mixtures of fungicides were tested in conditions which favoured the build-up of fire on unsprayed control plants. The full details and results are given in Appendix 9.

When the weekly assessments of fire incidence were summed as at Rosewarne, it was found that fortnightly spraying (starting in early March) of Murphane Wettable, Liromate and Bayer 3114 significantly decreased infection as did Polyram and tank-mix zineb but these latter two were less effective (Table 51). In this experiment yields were inversely related to the incidence of disease (Fig. 19) - the regression coefficient of leaf disease on yield indicating a very close linear relationship ($r = -0.87$); the regression line in Fig. 20 is fitted to the data from separate replicate plots. The fungicide treatments fall into three distinct groups and their position may be taken as an indication of their efficiency in controlling disease.

Fig. 19. Effects of different fungicidal sprays on (a) incidence of B. tulipae and (b) tulip yields. (Kirton 1965/66)

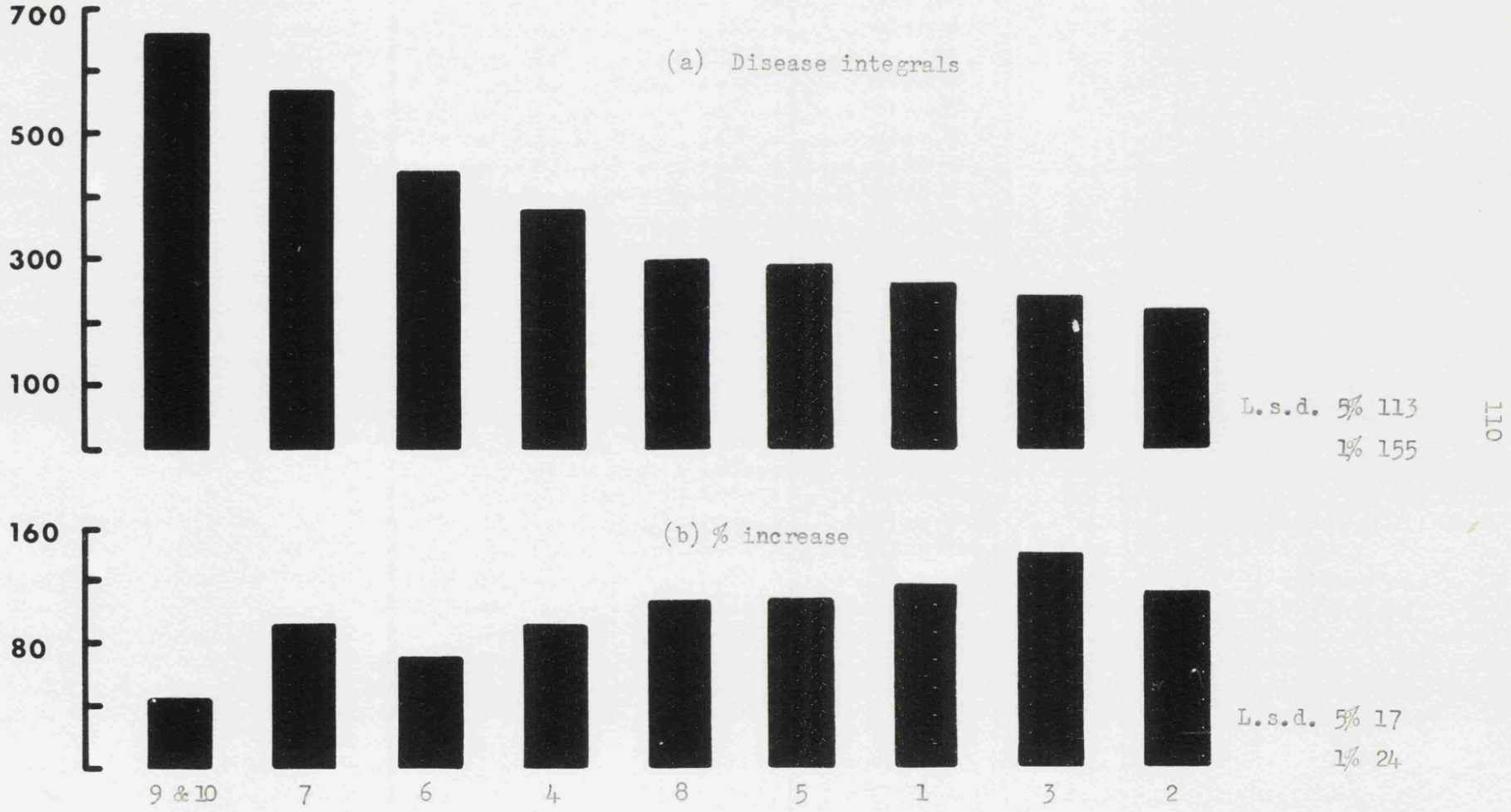


Fig. 20. The relation between yield and different amounts of leaf damage caused by B. tulipae (Kirton 1965/66)

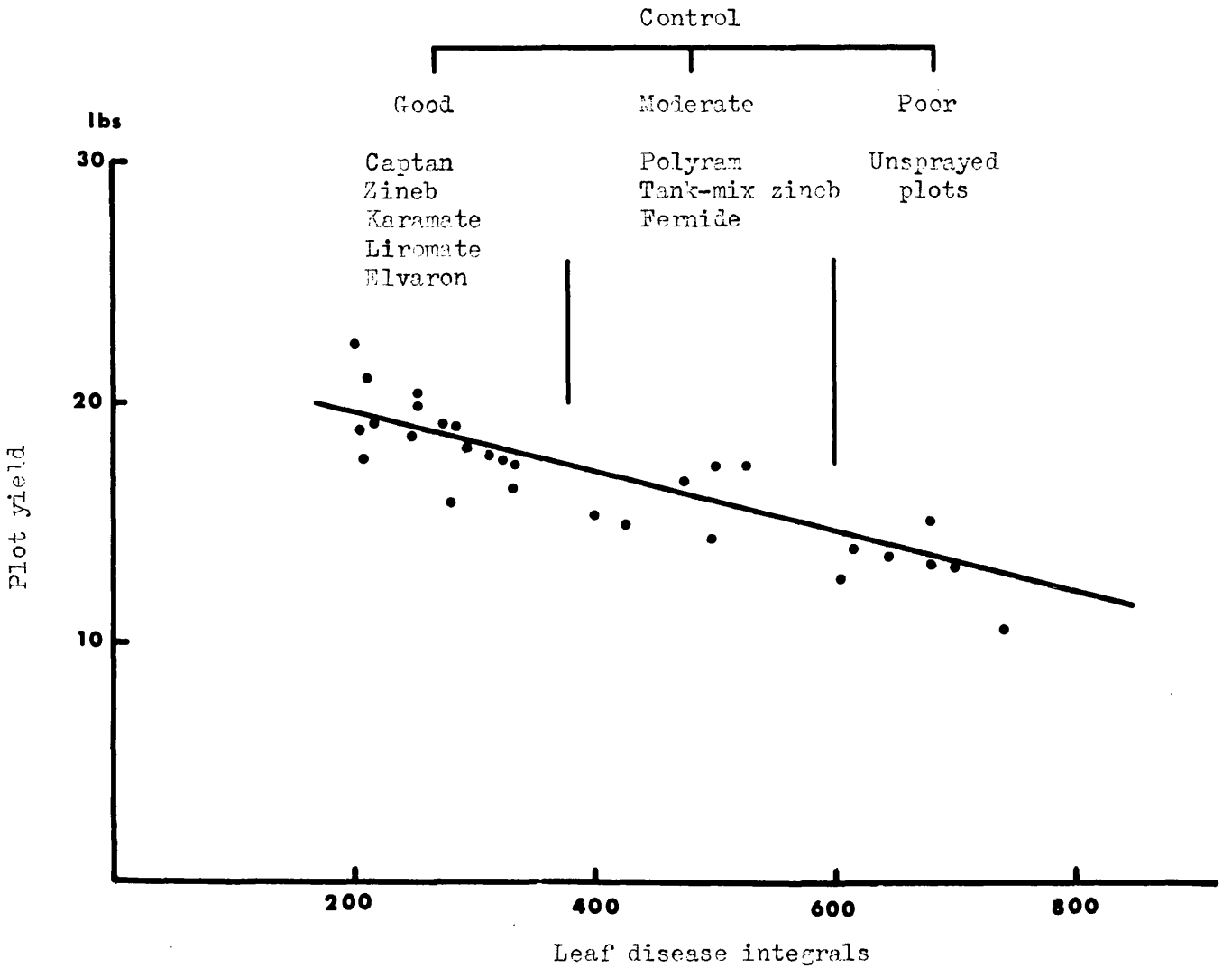


Table 51

EFFECT OF DIFFERENT FUNGICIDAL SPRAYS ON (A) INCIDENCE OF B. tulipae AND (B) YIELD OF TULIPS AT KIRTON 1966/67

Treatment	Disease integrals	% increase *
'Healthy control'	140	132
Dithane 945	194	132
Karamate	206	125
Elvaron 3 lbs	210	131
Murphane Wettable	234	90
Elvaron 2 lbs	242	123
Liromate	280	109
Tank-mix zineb	310	101
Polyram	314	85
Unsprayed control	517	40

* calculated as increase over planting weight

Results of 1966/67 at Kirton and Rosewarne E.H.S.

Rosewarne. To estimate the maximum possible yield increase in this experiment, again testing a range of fungicides, primaries were not planted in a set of replicate plots which were sprayed weekly with Dithane 945 (the fungicide that gave the greatest control of B. tulipae at Kirton in 1965/66). At Kirton, in the previous year, Polyram and Fernide had shown little promise and these treatments were discarded, as was the mixture of Orthocide and Murphane Wettable. Instead Karamate and Elvaron (previously Bayer 3114), which had been effective at Kirton, were introduced. The formulation

of tank-mix zineb was modified to include petroleum oil in an attempt to improve its fungicidal properties by increasing tenacity. (Walker 1967). The full details of this experiment are given in Appendix 9.

Although a primary was transplanted into the centre of each plot during early March, B. tulipae had not spread by early May and leaf assessments were discontinued. Nevertheless, significantly larger yields were obtained after using some fungicides. Dithane 945, Karamate and Murphane Wettable increased bulb yields from 107% in the unsprayed control plots to 138-147% (Table 52).

Table 52

EFFECTS OF FOLIAR SPRAYS ON DEVELOPMENT OF KRELAGE TRIUMPH BULBS IN THE ABSENCE OF B. tulipae AT ROSEWARNE 1966/67

Foliar spray	Lifted weight (lbs)	% increase *
Murphane Wettable	14.7	147
Dithane 945	14.6	145
Karamate	14.2	138
'Healthy control'	13.9	133
Elvaron 3 lbs	13.0	118
Elvaron 2 lbs	13.0	118
Tank-mix zineb	12.8	115
Unsprayed control	12.3	107
L.s.d. (p = 0.05)	1.22	
L.s.d. (p = 0.01)	1.70	

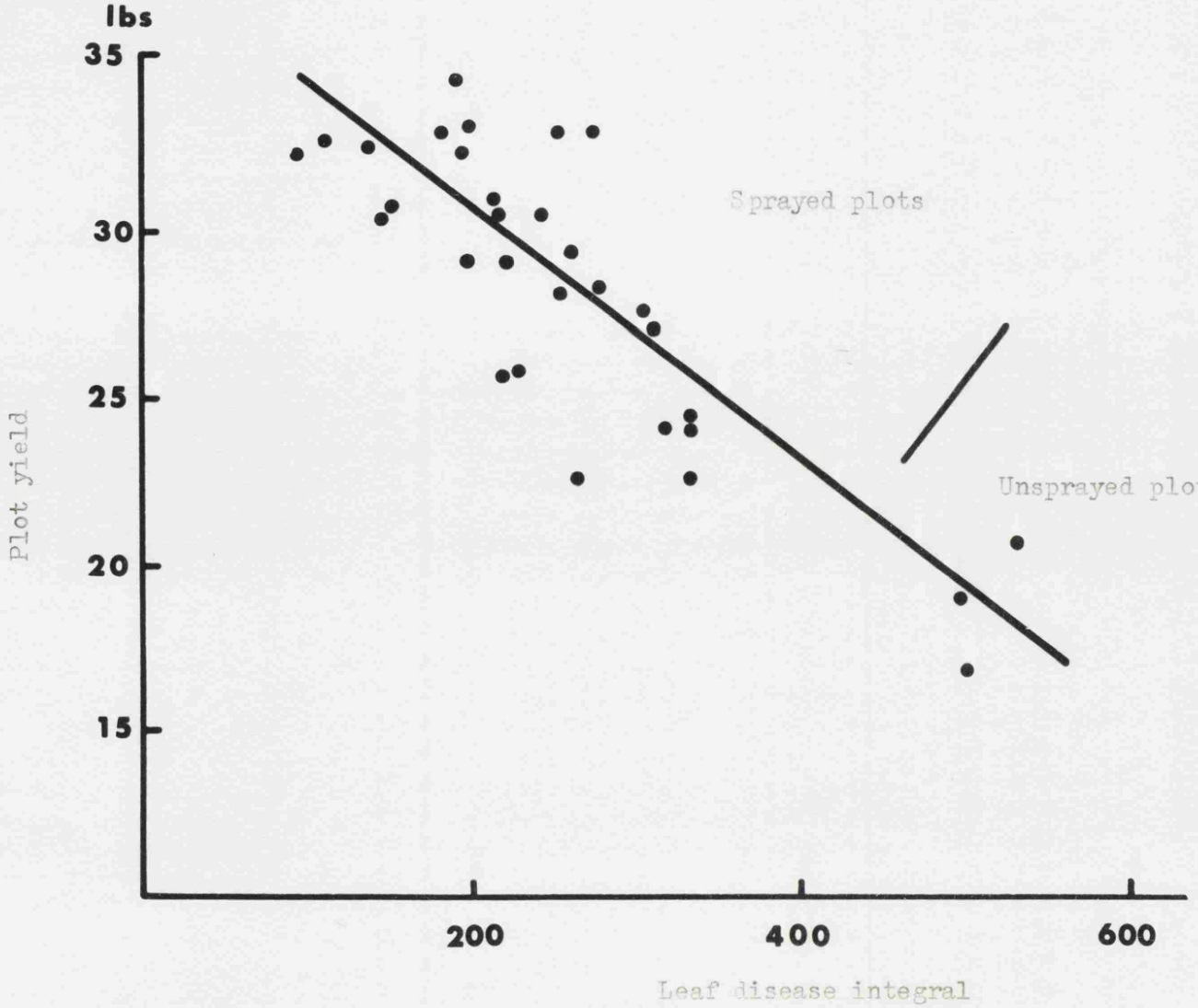
* calculated as increase over planting weight.

Casual observations suggested that the larger yields were associated with delayed leaf senescence after spraying with proprietary formulations of dithiocarbamates. Could this be attributed, not to internal factors, but to changes in the phylloplane microflora? Microbes, not usually considered to be pathogenic might, by continually eroding cuticle, cause damage which increased water loss and hastened senescence in the warm summer weather of Cornwall.

Kirton. As at Rosewarne, to estimate the maximum possible yield increase, primaries were not planted in one set of replicate plots which were, nevertheless, sprayed weekly with Dithane 945. The previous year's results showed that Karamate and Murphane Wettable were more efficacious than the other fungicides; the larger yields may simply reflect the greater fungitoxicities of their active components, i.e. the manganese-zinc complex of mancozeb and the zinc ethylene 1-2 bisdithiocarbamate, linked in the former with greater amounts of active ingredient. The Bayer 3114 formulation, released as Elvaron, which had been effective with a low dosage, was the one formulation differing markedly from the dithiocarbamate and was included because of this feature. Because of the limited space available Fernide and Polyram were deleted making way for a more tenacious formulation of tank-mix zineb with added petroleum oil. The full details and results are given in Appendix 9.

In the unsprayed plotswith infectors, the build-up of tulip fire was similar to that of 1965/66. By the end of the season spray treatments greatly influenced the incidence of disease, yields and leaf disease being related inversely (Fig. 21).

Fig. 21. Association of leaf disease integrals with bulb yields when tulip crops were protected by different fungicides from attack by B. tulipae at Kirton E.H.S., 1966/67.



Polyram and tank-mix zineb spray decreased the incidence of B. tulipae as compared with unsprayed plants but were less effective than other fungicides tested. Although the higher dose of Elvaron (3 lbs) gave the greater control of fire, the improved yield was unlikely to warrant the additional cost. Bearing in mind that the weekly sprayed plots were without infectors, decreasing the spraying interval of Dithane 945 from two to one week was unrewarding.

(e) Discussion. Prior to 1965 increased yields of tulip bulbs associated with the use of fungicidal sprays were usually attributed without further thought to the control of B. tulipae. But results obtained at Rosewarne in 1965/66 and 1966/67 cast doubt upon this interpretation and indicate the need for detailed observations during the growing season. For example, in 1965/66 some sprays appreciably decreased amounts of leaf infection but these effects were not reflected in corresponding yield differences, whereas in the next season substantially significant yield differences occurred in the absence of B. tulipae.

At Kirton interpretation of the results was straightforward, leaf infection and bulb yields being inversely related. Of all the formulations used those based onmancozeb (Karamate and Dithane 945) consistently gave high yields. Unfortunately the prolongation of leaf life, though apparently increasing yields, delays lifting - sometimes to the disadvantage of growers because of the clash with other agricultural operations. Elvaron is a good fungicide but is 22% more expensive than Karamate, a small increase relative to the value of the crop. Nine applications of Karamate cost c. £21.6 and of Elvaron £26.4 per acre respectively. According to the cultivar, bulbs costing between £500 and £1200 are needed to plant an acre,

yield increases vary with cultivar, spacing and season but values of 100 - 120% are common and the saleable fraction is usually equal or greater than the value planted.

It is unfortunate that bulbs planted at Kirton differed in grade in 1965/66 and 1966/67, because potential increase varies with grade (Rees 1969) and mixed grades within an experiment may cause misleading results even though the weight of bulbs planted per plot were equal.

The four field experiments have aided the selection of efficacious fungicicide and have indicated some of the problems when attempting to associate yield with foliar disease. The relation between arbitrary leaf disease scores and actual areas affected needs to be determined.

The amount of leaf spotting in tulip crops is governed by numbers of primaries, the dispersion of conidia, the efficiency of spraying and the effectiveness of the fungicide used. Spraying is usually done by tractor-drawn machines which, unfortunately, compact soil between ridges so creating a serious lifting problem at bulb harvest. How then, can the loss of yield through leaf spotting be equated against the harmful effects of soil compaction by spraying machinery? Complete control of fire may be neither desirable nor necessary, and some measure of leaf loss may be tolerable.

At the present time it is usual to assess the effects of fire at the end of the season by comparing unsprayed and sprayed plot yields. Fire is sometimes deliberately introduced into the experiments but its subsequent development depends upon the prevailing weather conditions and is unpredictable. Although providing valuable information about methods of spraying and

Plate 20.

Plate 20. Leaf pruning.

Unpruned plants.

12% reduction of leaf area.

40% reduction of leaf area.

64% reduction of leaf area.

comparisons of fungicides it is not possible, for example, to compare the effects of a late attack of fire with an early one, or mild with severe or indeed, any other combination.

Evidence has not been put forward that any Botrytis disease sequesters host-nutrients as do the rusts (vide Shaw M. and Samborski D.J., 1956). If the damage by B. tulipae were restricted to the areas of leaf actually invaded, then the effects should be, more or less, mimicked by cutting off equivalent amounts of foliage.

C. Simulation of leaf disease effects by leaf clipping.

Two experiments of this type were done, in the first residual mother bulb tissue and developing daughter bulbs were weighed on three occasions during the 1966/67 season and in the second, during the 1967/68 season daughter bulbs only were weighed. In order to test the reproductability of the leaf pruning treatments, areas, assessed by eye, and equivalent to 25%, 50%, and 75% of the length of laminae of each of the three leaves of five replicate plants were severed and subsequently measured photometrically. The data in Table 53 indicate that the areas removed were less than anticipated, but the decreases were reliably consistent. Removing approximately a quarter of the length of leaf laminae reduced leaf area by 12% with an extreme variation from 7 - 14% (Plate 20). The design and details of the two experiments are given in full in Appendix 10, the mean values for 1966/67 being presented in Table 54.

Analyses of these data gave the following results: The yield data indicate that (a) 64% loss of leaf area decreased bulb yields irrespective of the dates of leaf pruning and (b) losses of 12% did not affect yield. Together these suggest that amounts

Table 53

THE EFFECTS OF CUTTING OFF A QUARTER, HALF OR THREE-QUARTERS OF LENGTHS OF LAMINAE ON LEAF AREA

	Plant No.					Total	% reduction of area
	1	2	3	4	5		
Total leaf area	234	169	166	165	162	896	0
¼ length	202	143	144	150	151	790	12
½ length	125	100	104	104	109	537	40
¾ length	70	65	61	61	64	323	64

of fire are more important than the time of attack. As the experiment progressed, however, weed control deteriorated and weeds may have caused undue shading and competition.

An experiment with a similar set of treatments was planned for 1967/68 but with a different design, more efficient weed control and fortnightly spraying so that conditions would approximate to those in commerce. Yield assessments were made on daughter bulbs only: maidens and residues of mother bulbs being discarded (Table 55). The effects of leaf pruning done on one occasion progressively increased as the season advanced. Removing 64% of leaf area in March decreased bulb yields, measured 1 and 3 months later, by 30 and 46% respectively. Pruning done in March and April decreased yield during the subsequent months more than pruning done in May, by 30, 28 and 17% respectively, suggesting that early season infection with B. tulipae might be more damaging than late attacks. With some host parasite complexes small amounts of infection can cause disproportionately large decreases in the development of the host e.g., cereal powdery mildew (Large & Doling, 1962, Last, 1963).

Table 54

EFFECTS ON YIELD OF TULIP BULBS OF DECREASING LEAF AREAS BY DIFFERING AMOUNTS ON ONE OF THREE DIFFERENT OCCASIONS

1966/67

Leaf area decreases	Yield of bulbs (gm dry wt) sampled in		
	April	May	June
1. Leaves cut in March			
0	81.2	137.4	168.2
12	83.3	134.1	159.6
40	75.3	105.4	128.7
64	64.6	104.0	126.2
2. Leaves cut in April			
0		137.6	150.3
12		134.3	167.3
40		121.0	145.5
64		115.2	112.8
3. Leaves cut in May			
0			163.9
12			164.0
40			144.8
64			147.8
L.s.d. (p = 0.05)	18.2	14.2	25.8

Table 55

EFFECT ON YIELD OF TULIP BULBS OF DECREASING LEAF AREAS BY DIFFERING AMOUNTS ON ONE OF THREE OCCASIONS

1967/68

Leaf area decreases	Yield of bulbs (gm dry wt) sampled in		
	April	May	June
1. Leaves cut in March			
0	31.7	180.5	216.7
12	31.9	177.2	229.5
40	26.7	171.9	176.0
64	22.2	144.2	116.5
2. Leaves cut in April			
0		180.5	216.7
12		177.6	214.0
40		162.3	166.0
64		130.5	128.3
3. Leaves cut in May			
0			216.7
12			216.8
40			186.0
64			180.0
L.s.d. ($p = 0.05$)	4.6	16.5	25.2

But the results of this experiment suggest that tulips can tolerate leaf losses of c. 12%. Similar results have been reported by Compton (1960) in an investigation on the effects of atmospheric fluorine pollution on gladioli, who found that corm yields were not affected until 4 or more inches of leaf were removed, equivalent to 10% of leaf area.

If it were possible to consider above and below-ground phases of tulip fire in isolation, it would seem that yield is unlikely to be affected by the potentially more damaging early attacks since the incidence of B. tulipae rarely exceeds 12% during March and early April, even on unsprayed controls. Nonetheless, it is essential to cover foliage adequately by late April and May, and with our present technique this necessitates a series of early spray applications to protect leaves and flowers and to minimise the amounts of inocula which may infect developing daughter bulbs when washed down flower stems.

D. Other methods of control.

Tulip fire will be only partially controlled by foliar fungicides unless they are systemic because contact materials are unable to penetrate to the underground parts. Other methods are therefore required if primary sources of inocula are to be eliminated. Within the present system of crop husbandry there are several possible methods which are worth considering viz. dipping, fumigating and heat treating bulbs.

(a) Bulb dipping. Within the bulb industry fungicidal dipping is commonly practiced e.g., to control Narcissus basal rot, when formalin is added to the hot water used to control nematodes. Because tulips are often grown in conjunction with narcissi the apparatus necessary for dipping tulips is readily available.

Newton, Hastings and Bosher (1932) recorded that Canadian growers dipped tulips although few were convinced of its efficacy. To provide clear cut evidence, sclerotia produced *in vitro* were immersed in different chemical solutions, which were also watered on to pot-grown tulips. Among the chemicals used were formalin, mercuric chloride and copper sulphate and by recording concentrations toxic to sclerotia yet not harmful to tulips, they selected treatments for larger scale trials. Dipped bulbs were then planted in sclerotia infested soils during the autumn and numbers of diseased bulbs recorded next spring. Counts showed that formalin was less effective than mercuric chloride, although the overall amount of disease was small. Beaumont *et al* (1936) tested a similar range of chemicals but their results were inconclusive.

The effects of dipping treatments, as far as is known, have been based upon the subsequent appearance of above-ground disease symptoms. An experiment was started at Kirton E.H.S. in 1966 before the significance of underground phases of fire was understood. Four replicate batches, of equal weight, of 200 bulbs from a commercial stock were dipped for 30 min in suspensions containing proprietary formulations of 2% tecnazene, 1% mercuric chloride or 1% dicloran. Subsequent disease incidence was uniformly low *c.* 2% without being affected by treatment.

In the hope of obtaining a more stringent set of conditions, artificially inoculated bulbs were used in 1967. Because it was thought that *inoculated* bulbs would ensure a high incidence of fire, at least in the untreated controls, numbers of bulbs per treatment were decreased to replicate lots each of 50 instead of 200. Additionally four other replicate batches of

200 uninoculated bulbs per treatment were dipped for tests of phytotoxicity. Replicate batches of inoculated bulbs planted in October, were lifted in April, May, June and July and examined for disease development. The average infection on bulbs sampled on the first three dates of lifting was 18%, with disease incidence varying greatly among replicate batches. The results from the fourth batch, being assessed on newly developed daughter bulbs, were equally inconclusive. It was thought, in retrospect, that the dry conditions of storage after inoculation might have influenced disease development.

(b) Bulb fumigation. Bulbs lifted and graded in late July are usually stored until planting in October. To trigger flower development they are kept at c. 20°C during the initial stages of storage, subsequently being cooled at c. 10°C. Most bulb stores are well-built, insulated buildings, many having (a) forced draught ventilation and (b) heating facilities for preparing bulbs for forcing. This type of purpose built structure could be readily modified as a fumigation chamber if there were suitable fumigants for the control of bulb pathogens.

Because Jarvis (1967) found that sulphur dioxide controlled strawberry rots, caused by B. cinerea, the effect of this material against B. tulipae was examined. Bulbs inoculated with malt agar discs of B. tulipae culture, placed on fleshy scales, were exposed to three atmospheric concentrations of SO_2. Each fumigation treatment was tested on eight diseased William Pitt and 10 healthy Elmus tulip bulbs. A large dessicator of known volume, fitted with inlet and outlet pipes and a large polyethylene syringe, which distributed the gas uniformly when pumped, formed the chamber. The volume of SO_2 flowing at predetermined pressures

was measured using a manometer and capilliary tube mounted in parallel, the gas being collected over SO_2 saturated paraffin in an inverted burette.

After fumigation in July, bulbs were not planted until October when those exposed to SO_2 were soft, gas damage being suspected. In late November, when shoot emergence and root formation should have commenced, bulbs were lifted and examined. Most bulbs were dead but some survived short exposures to concentrations of 5 and 10% SO_2 (Table 56).

Table 56

NUMBERS OF BULBS SURVIVING IN NOVEMBER AFTER BEING FUMIGATED IN JULY WITH DIFFERENT CONCENTRATIONS OF SULPHUR DIOXIDE FOR DIFFERENT DURATIONS

SO^2 % concentration	Duration (min)	Nos. surviving (of 18)
5	5	3
5	15	0
5	30	0
10	5	4
10	15	0
10	30	1
20	5	0
20	15	1
20	30	0
untreated control	-	18

Plate 21.

Plate 21. 'Blindstoken'. Partial destruction of tulip flowers by heating bulbs for 2, 3 and 4 weeks at 30°C in August.

(c) Heat treatment. Experiments with B. tulipae suggested that the thermal death point of mycelium was between 30 and 35°C Valaskova (1963a), who exposed sclerotia at 35°C for periods of 1 to 4 weeks, subsequently culturing and incubating them at 20°C, found that viability was lost after 3 weeks.

For some years a few dry-bulb producers in the Netherlands have heated tulips to destroy the developing flowers within the bulb and obtain increased yields ('blindstoken' - Plate 21). Hartsema and Luyten (1950) tested several temperature regimes hoping to place the technique on a firm basis but they met with little success. By a coincidence Toyada and Nishii (1957), in experiments to induce early flowering, noted that some treatments prevented flower production with a concomitant greater development of daughter bulbs. Rees (1966) concluded that the discrepancy between the two sets of workers might be attributed to differences in bulb development at time of treatment, and recommended a 4-week exposure to a temperature of 33°C during August.

The separate results obtained by a pathologist, Valaskova, and a physiologist, Rees, hinted at the possibility of freeing stocks of bulbs from B. tulipae, with a treatment that would also favour greater bulb increments at the expense of flower development. In July 1967 , after removing the tunics of 10 cm Rose Copland bulbs, fleshy scales were inoculated with discs of malt agar containing B. tulipae mycelium. One month later these bulbs were arranged in 'Netlon' in 8 groups of 10 together with a duplicate series of uninoculated bulbs and then incubated at 33°C. At weekly intervals 10 healthy and 10 diseased bulbs were removed and kept at ambient temperature, with exposures ranging from 0 to 7 weeks. All the bulbs were planted in October and were subsequently

lifted in early April and May. The incidence of bulb infection was not controlled, 92% of the inoculated bulbs being attacked irrespective of heat treatment.

At the same time as bulbs were being exposed at 33°C, naturally produced sclerotia dissected from bulbs and subsequently kept in specimen tubes were also incubated at this temperature. Immediately after their removal, at weekly intervals, they were surface sterilised in hypochlorite, and incubated on malt agar at 20°C; all remained viable.

The temperature used by Valaskova to kill sclerotia was 2°C higher than that recommended for tulip flower destruction (33° C) although Toyada and Nishii (1957) found that tulips could tolerate higher temperatures (up to 35°C). Bulbs and isolated sclerotia were prepared and incubated as before but instead of being planted, lesion tissue was dissected from them and cultured on malt agar. After 4 weeks' exposure B. tulipae was still isolated from inoculated bulbs but the fleshy scales were browning and wrinkling. Sclerotia retained a degree of viability (Table 57), 12% remaining viable after 7 weeks' exposure, so the idea of freeing bulbs from B. tulipae by heat treatment was abandonned. Why was Valaskova able to show reduced viability of sclerotia exposed at 35°C? The discrepancy may be attributable to the differing physiological responses of naturally and artificially produced sclerotia.

(d) Discussion. The results show that heat treating bulbs, to free them from fire, is useless but that there may be merit in other methods. As a technique fumigation possesses many advantages because disease control would take place with virtually no dislocation of present cultural operations, and could be done at any

Table 57

NUMBERS OF SCLEROTIA STILL VIABLE AFTER BEING EXPOSED TO DRY HEAT (35°C) FOR DIFFERENT PERIODS

Exposure (weeks)	Number viable (of 25)	% viable
1	16	64
2	17	68
3	3	12
4	11	44
7	3	12

time during bulb storage. Much experimental work needs to be done, gases possibly being more penetrative than liquids to deep seated sites of mycelial infection. Bulb dipping is not so promising because of its more involved procedures, and unless very successful, it would have little appeal for growers. Without added wetters fungicidal solutions are unlikely to be as penetrative as a gas and they would entail subsequent drying. Furthermore, the fungicide would be sufficiently persistent to kill sclerotia when they germinate after the bulbs are planted many weeks later.

In spite of these difficulties both fumigation and dipping are worth pursuing because their success promises long-term control from one season to the next as opposed to the short-term single season control given by foliar sprays. Further, reducing or eliminating movement by spraying machinery would decrease soil compaction and make lifting easier.

DISCUSSION

The work described in this thesis was started primarily because tulip growers, notwithstanding regular rogueing and fungicidal spraying, were unable to reduce the annual incidence of "primaries" caused by B. tulipae. This suggested that our knowledge of tulip fire etiology was incomplete and it is now known that they were concentrating unwittingly on aspects of cure instead of prevention. Although prematurely emerging infected shoots usually give the first warning of B. tulipae attack, these "primaries", which are misnamed, are not part of the initial phase of the B. tulipae infection cycle. They are secondary manifestations of bulb infections occurring during the life of the preceeding crop. The development of the pathogen in these primary infections ceases temporarily during summer only to recommence growth shortly after planting in winter. Knowledge of the mechanism controlling this renewal of activity may help in seeking different and more efficient methods of disease control than those now in use.

Most of the thirty four generally accepted species of Botrytis are primary parasites with limited host ranges, with the notable exception of B. cinerea. Furthermore, most cause leaf lesions and in this respect B. tulipae conforms to the usual pattern. It would have been surprising if B. tulipae were unable to infect tulip bulbs which, in effect, are groups of swollen leaf bases. Although the role of soil-infesting sclerotia in the epidemiology of the disease is still to be defined it seems that the elimination of perennating bulb infections would break, in the absence of alternate hosts, the

cycle of spread. In the past, severely infested bulbs have been rogued before planting but discarding bulbs with obvious sclerotia is an inadequate measure because hyphae in slightly discoloured lesions on fleshy scales, often obscured by the tunics, are also able to initiate the build up of B. tulipae after planting. Our acquired knowledge of bulb infections now suggests that attempts at control should be concerned primarily with this phase of the complex and not with above-ground aspects as in the past. However, the damage done by infections established on flowers and aerial foliage, with associated yield decreases, should not be overlooked.

Very little is known of the occurrence of B. tulipae on wild tulip species in Asia Minor, Iran, Turkestan and Afghanistan, their centre of origin. Native tulips east of Turkey usually occur singly, at some distance from one another, suggesting that survival from one season to the next is by seed. In Turkey, however, the commonest types appear to be segregating forms of the sub-section Gesnerianae with dense clumps sometimes occurring, suggesting that in this locality vegetative propagation plays a part in perennation. Plant collectors and taxonomists seeking tulips have in the past probably unintentionally selected healthy and vigorous material but, even so, if aerial structures were commonly diseased the fact would have been known. The absence of such information suggests that wild tulips may be 'resistant', escaping severe infection possibly because of their sparse distribution. On the other hand, can it be assumed that leaf structures forming storage organs, which remain below ground will respond to infection in the same way as those emerging above ground and becoming photosynthetic? Aggressive infections on

above-ground foliage commonly produce abundant conidia but the production of the latter has not been observed when sampling buried infected bulbs. Lesions on bulbs frequently bear sclerotia and experiments done *in vitro* have shown that high carbohydrate media favour their production. Nonetheless, natural bulb infections are often found without sclerotia of *B. tulipae*. In the United Kingdom, the above-ground phase of the disease is commonplace and the underground phases have passed unnoticed for many years. It is possible that *B. tulipae* might, without our knowledge, be confined to the underground organs of tulips in their native habitat. Alternatively, some species may be genuinely resistant, this quality so far not being recognised by collectors of new species. If this latter possibility exists more rational use might be made of resistant species in the present programme for breeding attractive flowers which are, unfortunately, usually susceptible to *B. tulipae*.

The damage done by inocula carried on infected bulbs depends upon the differing responses of host and pathogen to soil temperatures, classical primaries being less likely to form in severe than in mild winters. Similarly, the development of foliar infections triggered by air and water-borne conidia depends upon the intricacies of the host's response to attack. As with *B. fabae* attacking *Vicia faba* L. (Deverall, 1960) it appears that some *B. tulipae* infections can be restricted, possibly by the production of phytoalexins, if the amount of inoculum is not too great and if prevailing weather does not favour the continuing persistance of infection drops or films.

In the main bulb areas of the United Kingdom tulips are usually grown in a 5 -course rotation. If, as the evidence suggests, bulb infections are the main source of carry-over such a cultivation system would seem unnecessary if planned solely for the control of B. tulipae, because ground-keepers are rarely seen to survive more than one season. The most important section of this thesis, dealing with the transmission of B. tulipae from mother to daughter bulbs, suggests that control measures should be re-orientated. Even if disease resistant tulip species were found, the production of suitable hybrids would take many years and other means of long term control should be sought. In the immediate future growers would be advised to continue with even more careful rogueing, fungicide spraying and selection of healthy plant material. Heat treatment would seem to be useless, and although preliminary fumigant treatments were not promising refinements in technique and choice of gas might lead to the control of the pathogen during bulb storage. Another possibility is the use of systemic fungicides. Benlate, a proprietary formulation of methyl 1 - (butylcarbamoyl) - 2 - benizimidazole carbamate, which is absorbed through roots, may be worth testing as it is claimed to decrease the incidence of B. cinerea on a range of hosts.

Numerous species of Botrytis attack bulbs and in due course tests will be made to see if there are important bulb phases in the etiology of B. narcissicola Kleb. and Sclerotinia polyblastis Gregory attacking narcissus. Not all Botrytis species with narrow host ranges attack leaf laminae: B. anthophila Bondartzev lives systemically within the clover plant and B. calthae Henn. and Elliott attacks petioles of Caltha palustris L.,

only penetrating leaves after their collapse. The initial infections of paeony shoots in spring by B. paeonie Oudem. takes place at ground level which suggests over-wintering of the pathogen below ground. Like B. allii Munn attacking onions, B. tulipae invades leaves and bulbs although, unlike the former, it does not seem to live systemically within the host. While doing the work described in this thesis, cursory examinations of ink disease of iris caused by Bipolaris iridis (Oudemans) Dickinson suggested that the type of etiology described for tulip fire is not restricted solely to complexes involving Botrytis species.

Plate 22.

Plate 22. Arrangement of soil tensiometers in a ridge planted with tulips.

Appendix 1

The measurement of soil moisture near bulbs planted in ridges.

If a porous pot, full of water, is placed in soil which is at field capacity the water will remain in the pot, but if the soil dries, water will flow into the soil. If the porous pot is connected to a closed system the water movement will create a partial vacuum, which is measurable and termed 'soil tension'. A common method for measuring soil tension is to connect the water reservoir in a porous pot with capilliary tubing to a mercury reservoir so that the movement of the mercury in the capilliary tube, caused by water movement, is measurable.

The apparatus used consisted of 18 tensiometers buried at bulb depth in a ridge planted with tulips. Each tensiometer (Fig. 22) was made by connecting a small porous pot and a wide nylon tube to a mercury reservoir with nylon capilliary tubing - the mercury being contained in a small test tube. Near soil level a nylon 'T' piece was inserted between the nylon tube and the capilliary tube, normally sealed off, enabling the system to be filled and kept full with sterile, boiled distilled water. The arrangement of the apparatus is shown in Plate 22. The battery of mercury reservoirs (not visible in the plate) was mounted on a board firmly fixed in the ground. Measurements of heights of the mercury columns were made daily at 0900 hr on weekdays. Not all the tensiometers were operative all the time but weekly mean values, based upon a 5 day week, were calculated from at least 55 measurements. The method of calculating soil tension was that used by Rutter (1964) i.e.

$h_1 (s - 1) - h_2 = T$ cm water, where h_1 = height of mercury column,
h_2 = height of mercury reservoir from porous pot, and
s = specific gravity of mercury at 20°C.

The weekly mean values are given in Table 58, and Fig. 23, which show an erratic but continuous increase in soil tension as the ridge became drier in summer.

Table 58

CHANGES IN SOIL MOISTURE CONTENT IN A BULB RIDGE MEASURED AS NUMBER OF CENTIMETERS OF WATER REQUIRED TO BRING THE SOIL TO FIELD CAPACITY

Week ending		Soil tension (cm water)
April	1 - 5	3.8
	8 - 11	16.1
	16 - 19	5.3
	22 - 26	3.8
April/May	29 - 3	1.3
	6 - 10	3.3
	13 - 17	6.3
	20 - 24	6.2
	27 - 31	19.6
June	4 - 7	13.8
	10 - 14	48.1
	17 - 21	68.7
	24 - 28	19.0

Fig. 22. Diagram of constructin of tensiometers and height measurements used in calculating soil tension in bulb ridges.

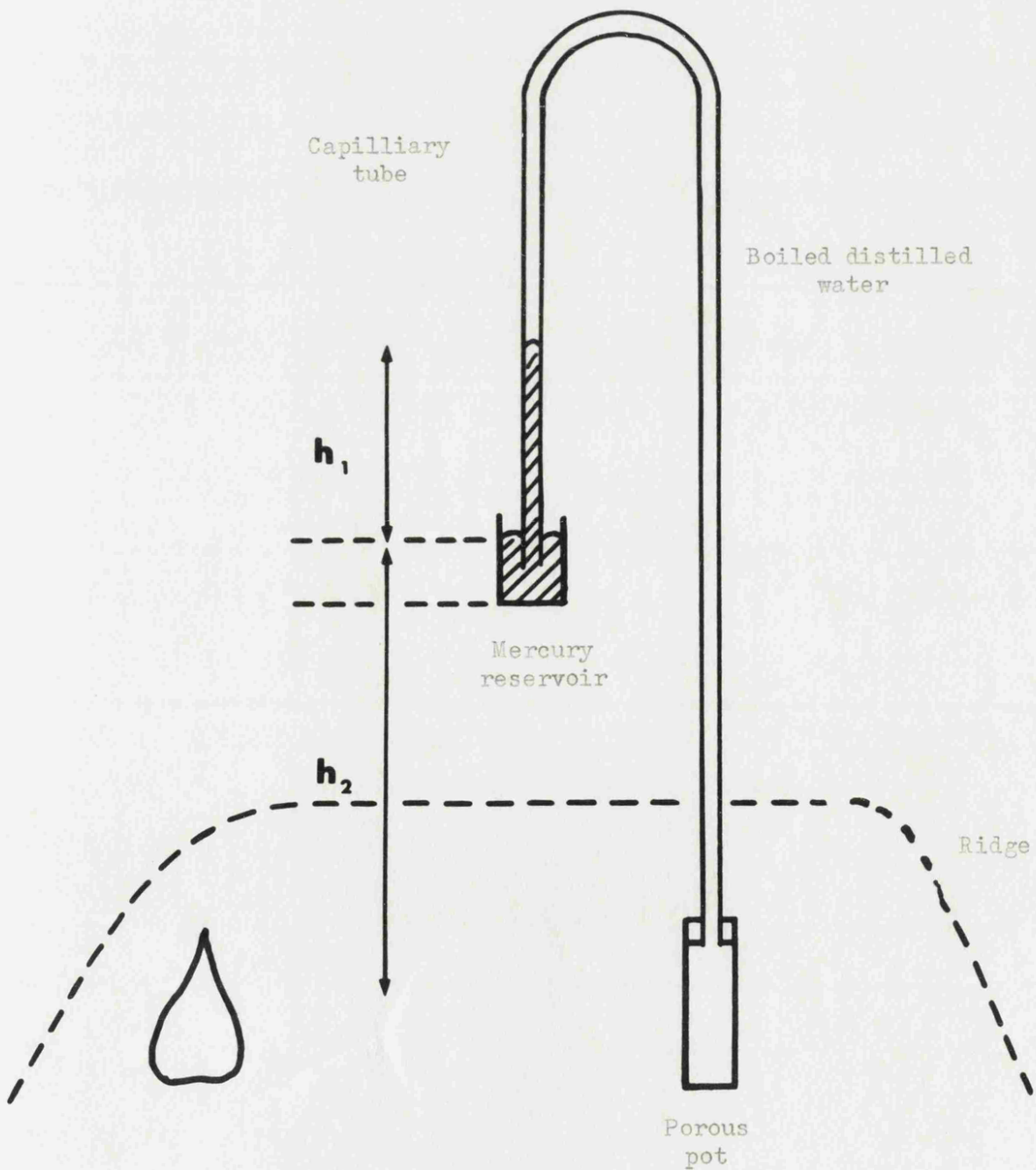
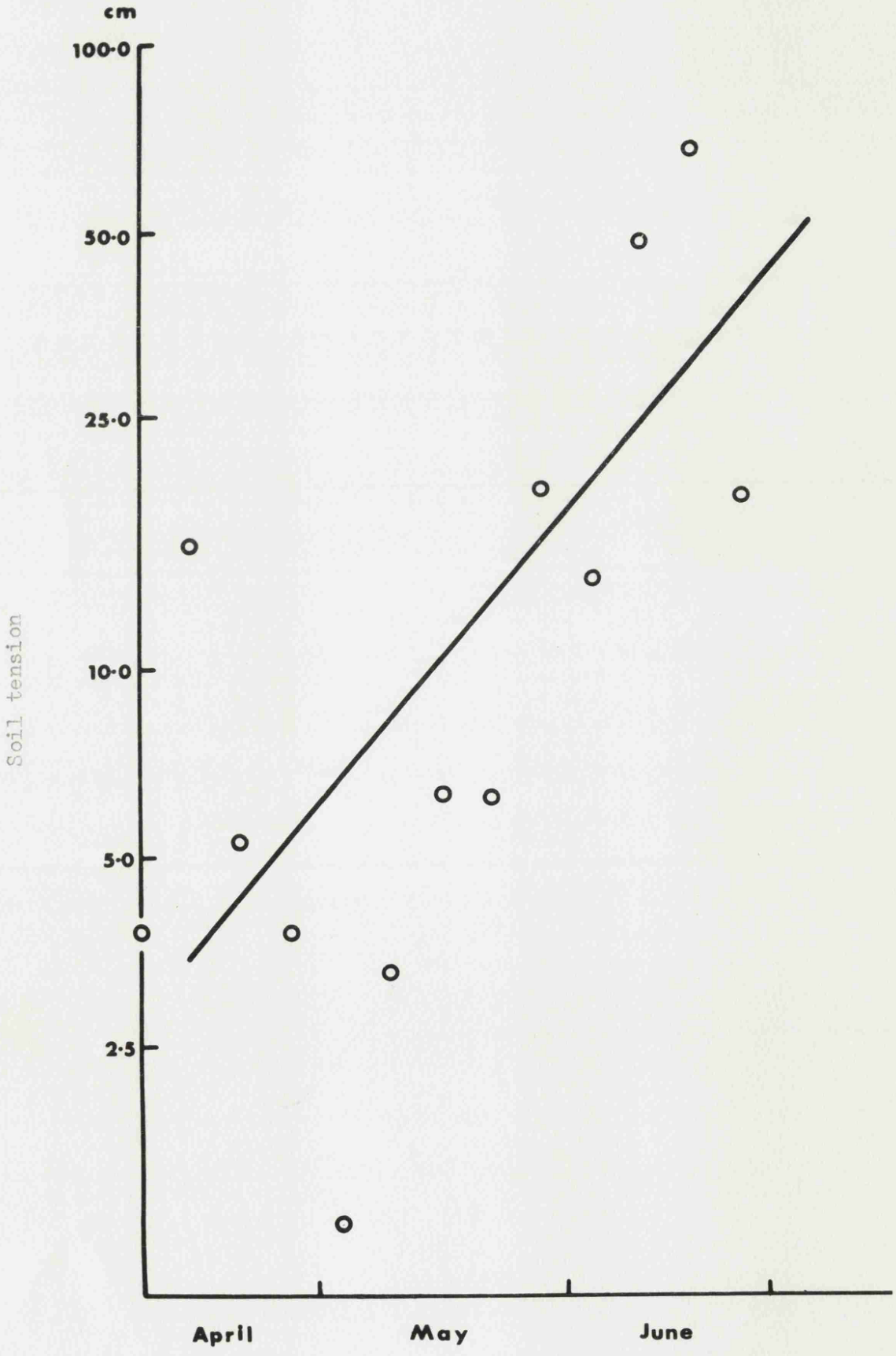


Fig. 23. Changes in soil moisture in tulip bulb ridge plotted as log. cm of water required to bring soil to field capacity.



The 'Split base' condition of tulips

In the past few years the condition of 'split base' has been observed repeatedly on cultivars of the Darwin hybrid group, including Apeldoorn, Dover, London and Oxford. When lifted, root plates were corky and fissured, more often than not with smaller than usual sclerotia of B. tulipae (Plate 6a). Although seriously affected bulbs would be easily detected, bulbs with slight symptoms would probably not be set aside during cleaning and grading operations and consequently they would be replaced.

Bulbs with split bases examined have all been first daughters, i.e. those with current seasons shoots attached at the base. It seems that if stem bases of mother bulbs become infected with B. tulipae when daughter bulbs are enlarging, part of the daughter's root plate is infected and killed; with this restriction and the continuing development of the rest of the bulb, cracks appear giving 'split bases' at maturity.

To see what happens when 'split base' tulips are cropped 32 ungraded Apeldoorn bulbs with this symptom were planted in boxes in November and placed out-of-doors. After shoots appeared, at the end of March, the bulbs were carefully washed and examined. Of 32 replicate bulbs, 8 were healthy, 2 severely colonised by Penicillium species, 1 developed as a tulip fire primary and 21 rotted basally with B. tulipae. The normal sequence of daughter bulb development did not occur in mother bulbs with split bases. Some of the outer daughter bulbs unexpectedly developed single leaves in a manner similar to the maidens forming from the outermost axillary bud beneath the tunic (Plate 6b).

Appendix 3

Comparative respiration rates of bulbs with and without B. tulipae infections

A non-destructive method for assessing respiration was used to measure acitivity of B. tulipae within lesions of fleshy scales of 'dormant' bulbs. Diseased bulbs were obtained by inoculating the outer fleshy scales of 10/11 cm William Pitt bulbs with 5 mm diameter discs of B. tulipae mycelium and then incubating the bulbs for a week at high humidity. At the end of this period, lesions about 1 cm diameter had formed and externally their enlargement seemed to have stopped. Using an apparatus similar to that of Birch and Friend (1956) the respiration rates of 5 diseased and 4 healthy bulbs were determined over nine consecutive periods, each of 24 hours.

The apparatus is, in essence, a crude Warburg respirometer with electrolytic integration (Fig. 24). Carbon dioxide released by the suspended tulip bulb (A) in the respiratory chamber (B) was absorbed by concentrated sodium hydroxide, and as a result of oxygen uptake during respiration the pressure within the apparatus falls. This partial vacuum caused the N sulphuric acid to rise in the anode arm of the electrolytic integrator and when it reached the anode at X the circuit within the acid reservoir (Y) was completed. As a result, oxygen was evolved at the anode to replace that used by the bulb in the respiratory chamber, and twice the volume of hydrogen released at the cathode (Z). The volume of oxygen corrected for NTP taken up by the bulb was half that of the released hydrogen which collected in an inverted burette.

The respiration rates of healthy and diseased bulbs (Table 59) were similar indicating that B. tulipae did not greatly influence bulb development at this stage in its life cycle. The considerable variation from bulb to bulb was shown to be commonplace by Rees (unpublished).

Table 59

RESPIRATION RATES OF TULIP BULBS INOCULATED WITH *B. tulipae*

Respiration measured in 1 O_2/hr/g fresh weight

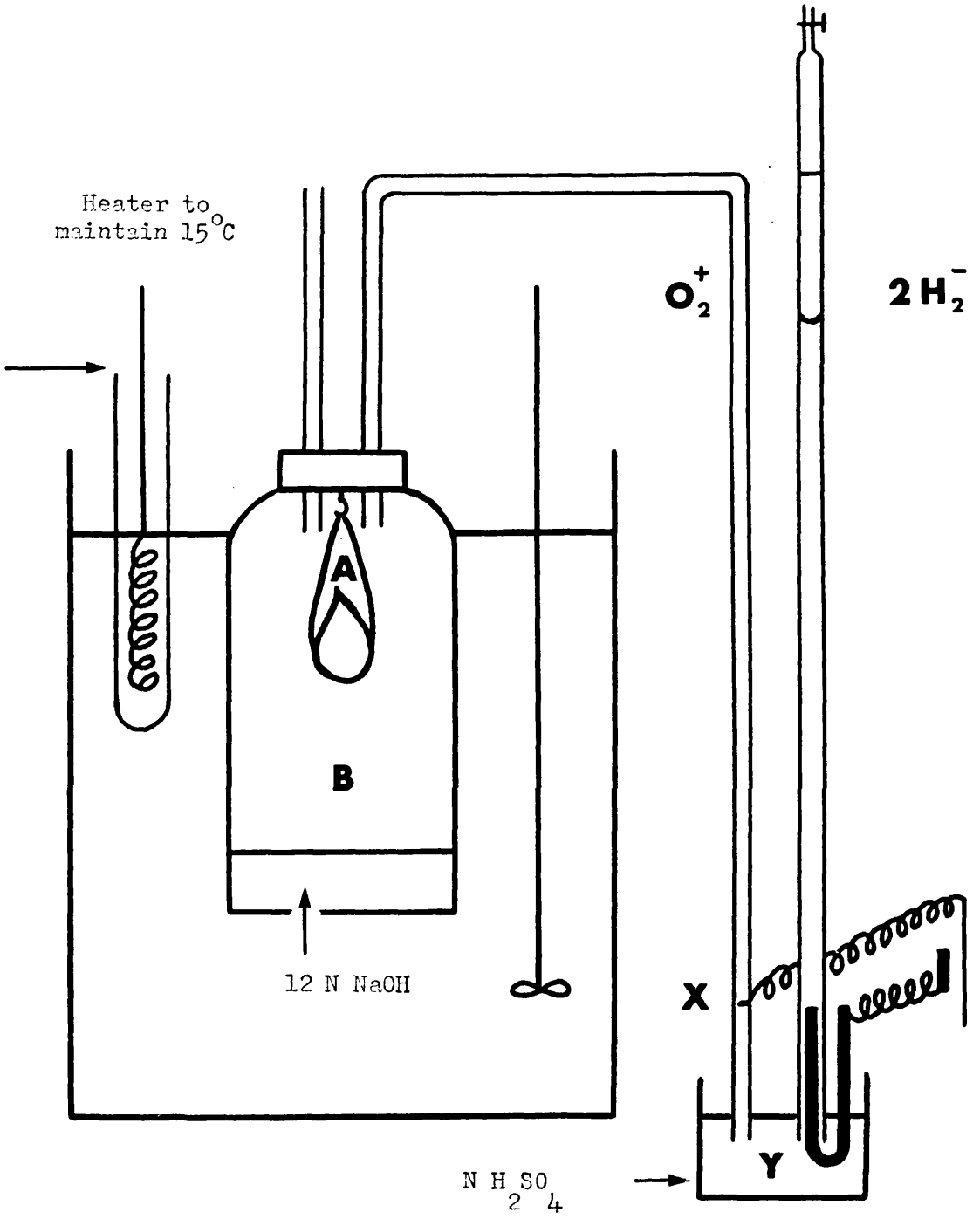
1. Healthy bulbs

	Replicate bulbs				
Period	1	2	3	4	Mean
1	8.3	15.4	14.3	11.7	12.4
2	12.6	16.4	16.6	10.0	13.9
3	13.2	13.0	15.3	10.0	12.8
4	11.2	11.0	11.4	8.9	10.6
5	10.0	9.0	10.2	6.0	8.8
6	12.3	11.5	14.5	10.2	12.1
7	11.5	11.8	10.9	10.6	11.2
8	9.4	6.8	11.3	5.8	8.3
9	10.5	8.3	14.0	6.3	9.8
Mean	11.0	11.5	13.2	8.8	11.1
S.E.s	±0.53	±1.29	±0.80	±0.75	

2. Diseased bulbs

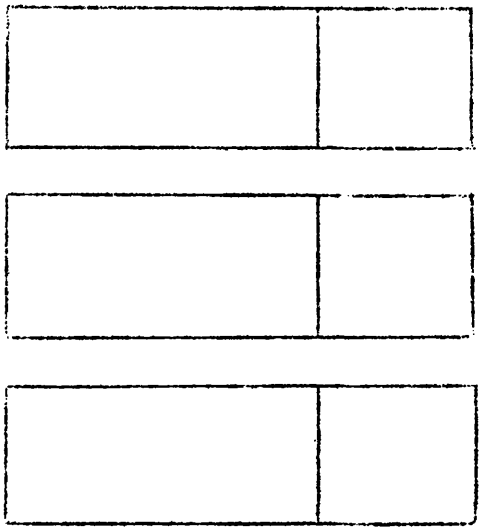
						Mean
1	5.2	12.3	16.5	14.3	17.9	13.2
2	9.4	13.1	14.6	12.7	15.8	13.1
3	10.0	12.0	12.0	12.5	14.5	12.2
4	7.4	9.9	9.7	8.9	11.6	9.5
5	6.0	8.9	8.2	7.8	9.2	8.0
6	7.4	9.4	11.1	10.0	11.7	9.9
7	7.0	9.6	8.1	8.0	11.1	8.8
8	3.6	7.1	6.2	5.3	6.1	5.6
9	5.9	8.3	8.0	6.8	8.2	9.7
Mean	6.9	10.1	10.5	9.6	11.6	9.7
S.E.s	±0.67	±0.67	±1.16	±1.00	±1.35	

Fig. 24. Apparatus for determining bulb respiration rates.



Appendix 4

The effect of cultivation and position of inoculation on the development of B. tulipae in bulbs, 1966/67



Cultivar and Grade : Rose Copland 10/11 cm.

Design and Cultivation : 10 bulbs in Netlon, 5 in apart. Bed planted bulbs planted 5 in deep; ridge planted bulbs planted 5 in from top of ridge in two rows per ridge 2 ft apart in late October.

Experimental treatments : Sclerotia of B. tulipae, isolate GCRI 6, from dried malt agar cultures inserted obliquely into

(a) fleshy scale at neck of bulb,

(b) fleshy scale at middle of bulb and

(c) uninoculated bulbs for control treatment.

Records : 1. 10 bulbs from each treament lifted fortnightly for examination.

2. Soil temperature at bulb depth in ridges and beds continuously recorded.

Table 60

EFFECTS OF DIFFERENT SITES OF INOCULATION AND CULTURAL PRACTICES ON THE DEVELOPMENT OF TULIP FIRE

Sampling period	Distribution of infection: Shoot not emerging: Mother bulb rotted	Shoot not emerging: Mother bulb rots: Shoot healthy	Shoot not emerging: Mother bulb rots: Shoot infected: Stem	Shoot not emerging: Mother bulb rots: Shoot infected: Leaf	Shoot emerging: No apparent spread from inoculation site
(a) Changes in expression of disease during the growing season.					
Planting - emergence	2	106	0	32	20
Emergence - heading	0	78	12	22	8
Heading - late May	4	110	24	20	1
Seasonal totals	6	294	36	74	29
(b) Total numbers at different sites of inoculation					
Middle of bulb	3	187	16	13	0
Neck of bulb	3	107	20	61	29
(c) Total numbers from different planting procedures					
Bulbs planted in Beds	3	154	19	22	21
" " " Ridges	3	140	17	52	8
(d) Totals for interaction between different inoculation sites and planting procedures					
Bed planted, middle lesion	2	92	8	7	0
Bed planted, neck lesion	1	62	11	15	21
Ridge planted, middle lesion	1	95	8	6	0
Ridge planted, neck lesion	2	45	9	46	8

Appendix 5

The effect of position of inoculum on the development of B. tulipae on bulbs planted in ridges, 1967/68

Neck infected bulbs	Middle infected bulbs	Lifting dates
19 fortnightly batches		6.11.67 to 21.7.68
		Final lifting 29.7.68

Cultivar and Grade : Rose Copland 10/11 cm.

Design and Cultivation : 10 bulbs in Netlon, 5 in apart. Bulbs planted 5 in from top of ridges in singles rows per ridge, 2 ft apart, in October.

Experimental treatments : Sclerotia of B. tulipae, isolate GCRI 6, from dried malt agar cultures inserted obliquely into

(a) fleshy scale at neck of bulb and,

(b) fleshy scale at middle of bulb.

Records : 1. 10 bulbs from each treatment lifted fortnightly for examination and a residual lifting at senescence.

2. Soil temperature at bulb depth continuously recorded.

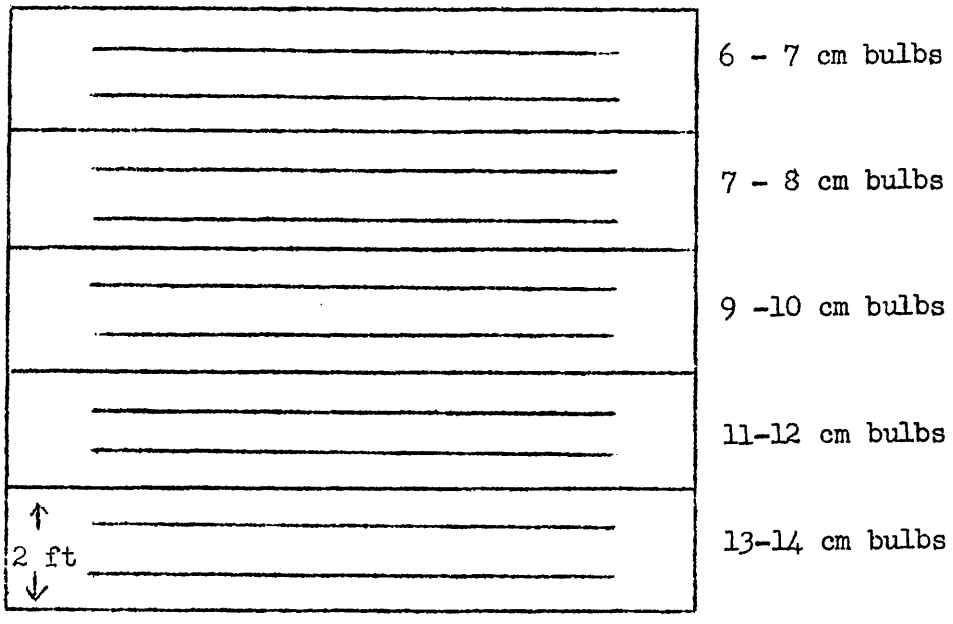
Table 61

EFFECTS OF DIFFERENT SITES OF INOCULATION ON THE DEVELOPMENT OF TULIP FIRE IN 1967/68

Sampling period	Distribution of infection: Shoot not emerging: Mother bulb rotted	Shoot emerging: Mother bulb rots: Shoot: healthy	Shoot emerging: Mother bulb rots: Shoot infected: Stem	Shoot emerging: Mother bulb rots: Shoot infected: Leaf	Shoot emerging: No apparent spread from inoculation site
(a) Changes in expression of disease during the growing season.					
Planting - emergence	1	98	3	0	38
Emergence - heading	6	32	8	1	73
Heading - senescence	7	6	27	0	40
Seasonal Totals	14	136	38	1	151
(b) Total numbers at different sites of inoculation.					
Middle of bulb	3	74	18	1	74
Neck of bulb	11	62	20	0	77

Appendix 6

Development of B. tulipae in artificially inoculated bulbs of different grades



Cultivar : Rose Copland

Design and Cultivation : 10 bulbs in Netlon 5 in apart. Bulbs planted 5 in from top of ridges in double rows 5 in apart in ridges 2 ft apart, in October.

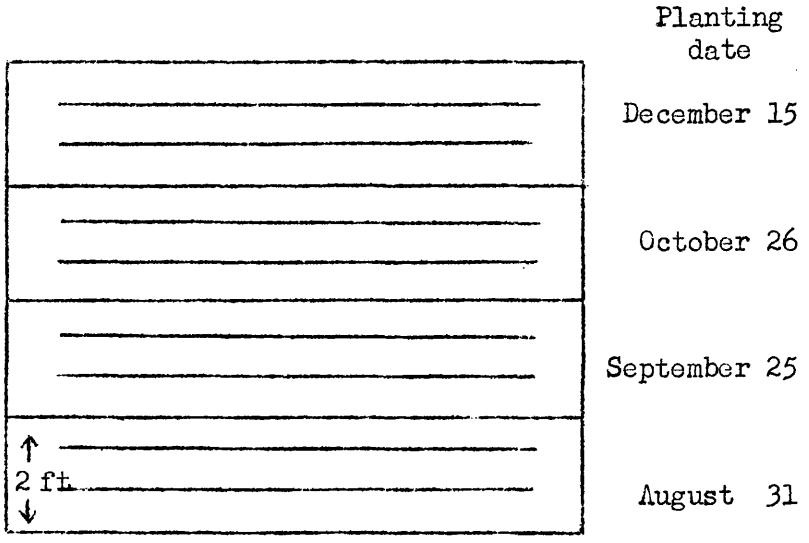
Experimental treatments : Sclerotia of B. tulipae, isolate GCRI 6, from dried malt agar cultures inserted obliquely into outer fleshy scales of necks of bulbs of five different grades.

Records : Numbers of primaries recorded on appearance and then cut off at ground level.

Bulbs lifted in June and examined for progress of disease.

Appendix 7

Effect of planting date on the development of B. tulipae in artificially infected bulbs



Cultivar and Grade : Rose Copland 10/11 cm.

Design and Cultivation : 10 bulbs in Netlon 5 in apart. Bulbs planted 5 in from top of ridges in double rows 5 in apart in ridges, 2 ft apart, in October.

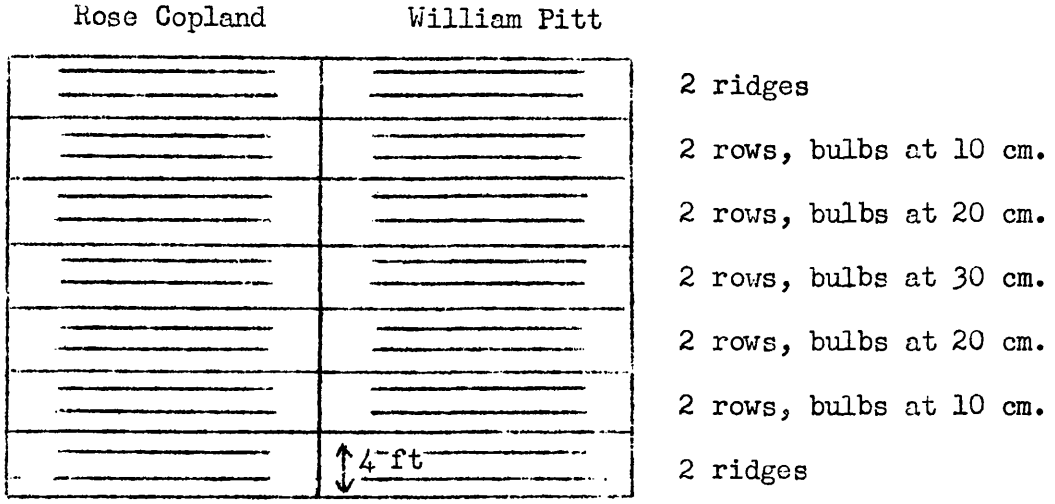
Experimental treatments : Sclerotia of B. tulipae, isolate GCRI 6, from dried malt agar cultures inserted obliquely into outer fleshy scales of bulbs planted on four different dates.

Records : Numbers of primaries recorded on appearance and then cut off at ground level.

Bulbs lifted in late May and examined for progress of disease.

Appendix 8

Effect of planting depth on the development of B. tulipae in artificially inoculated bulbs



Cultivars and Grade : Rose Copland and William Pitt, 10/11 cm

Design and Cultivation : 10 bulbs in Netlon 5 in apart. Bulbs planted at four different depths in double rows 2 ft apart in October, having been inoculated in August with B. tulipae sclerotia in the outer fleshy scales of the neck.

Records : Numbers of primaries recorded on appearance and then cut off at ground level. Half the experiment lifted in May and the progress of disease in the mother bulbs recorded, the other half being lifted at senescence and the progress of B. tulipae in the daughter bulbs recorded.

Appendix 9

Tulip fire control experiments

Rosewarne E.H.S. 1965/66

Replicate blocks				
I	5	7	3	8
	2	4	1	6
II	8	1	3	6
	4	5	7	2
III	6	4	8	1
	5	7	2	3

Cultivar : Krelage Triumph

Grade : 11/12 and 12/13 cm bulbs.

Design : 3 blocks each of 8 randomised plots. 200 bulbs weighing 14.74 lbs planted/plot in October in 2 x 12 ft long rows 2 ft apart.

Experimental treatments : (weights of fungicide/60 gal.)

1. Orthocide, 2 lb, plus Murphane Wettable, 2 lb (captan plus zineb, Murphy Chemical Co.)
2. Murphane Wettable, 2.5 lb (zineb, Murphy Chemical Co.)
3. Dithane 945, 4 lb. (mancozeb, Pan Britannica Ltd.)
4. Polyram, 2 lb (metiram, Boots Pure Drug Co.)
5. Tank-mix zineb (Dithane A 40 1.75 lb, zinc sulphate 1.25 lb, Pan Britannica Ltd., plus 10 fl. oz. Manoxol OT/A wetter in 60 gal.)
6. Fernide, 4 lb (thiram, Plant Protection Ltd.)

7 & 8. Unsprayed controls.

Weed control : Linuron and paraquat applied before bulbs emerged at makers recommended rates.

Fertilisers : None.

Cultural treatments : Primaries removed on appearance and one replaced in a central position in each plot in early March (Fig. 12A). Fungicidal sprays applied fortnightly at 60 gal/acre commencing mid-March.

Records : 1. Bulb weights at harvest.

2. Weekly leaf disease assessments on tagged plants.

RESULTS

Leaf disease assessments
(seasonal totals of disease integrals)

Spray treatments	Replicates			Mean value
	1	2	3	
Dithane 945	190	179	309	192.7
Polyram	238	230	180	216.0
Tank-mix zineb	226	194	233	217.7
Fernide	307	186	241	244.7
Orthicide plus Murphane Wettable	269	278	202	249.7
Murphane Wettable	303	229	260	264.0
Unsprayed control	241	350	287	292.7

Variance analysis

	d.f.	S.S.	M.S.
TOTAL	23	71067.33	
Blocks	2	2924.33	1462.17
Treatments	7	43422.67	6203.24
ERROR	14	24720.33	1765.74

Significantly different at 5% probability level using Snedecors F test.

Calculation of 5% l.s.d. $\sqrt{\frac{1766}{3}} \times \sqrt{2 \times t} = 73.52$

Yields of bulbs

(lbs/plot)

Spray treatments	Replicates 1	2	3	Mean yield
Dithane 945	19.3	18.2	18.8	18.8
Polyram	18.3	20.7	20.8	19.9
Tank-mix zineb	19.8	20.3	15.6	18.6
Fernide	20.0	16.8	20.1	19.0
Orthocide plus Murphane Wettable	19.9	21.4	20.6	20.6
Murphane Wettable	18.6	19.0	20.4	19.3
Unsprayed plots	18.6	17.6	17.6	17.9

Variance analysis

	d.f.	S.S.	M.S.
TOTAL	23	458.10	
Blocks	2	3.33	1.66
Treatments	7	150.40	21.49
ERROR	14	304.37	21.74

No significant differences

Kirton E.H.S. 1965/66

Replicate blocks

I		II		III	
9	6	9	5	4	2
5	3	7	1	7	3
4	2	6	8	10	1
7	1	2	3	9	6
8	10	10	4	5	8

Cultivar : William Pitt

Grade : Row 1 - 10/11 cm and maiden bulbs, Rows 2-5 - 10/11 cm bulbs.

Design : 3 blocks each of 8 randomised plots. 200 bulbs, weighing 8.75 lbs, planted in beds of 5 rows, each 8 in apart during October.

Experimental treatments : (weight of fungicide/60 gal)

1. Orthocide, 2 lb, and Murphane Wettable, 2 lb (captan and zineb, Murphy Chemical Co.)
2. Murphane Wettable, 2.5 lb (zineb, Murphy Chemical Co.)
3. Karamate, 3 lb (mancozeb with zineb, Pan Britannica Co.)
4. Polyram, 2 lb (metiram, Boots Pure Drug Co.)
5. Liromate, 4 lb (ferbam, Ligtermoet Chemie N.V.)
6. Tank-mix zineb (Dithane A 40 1.75 lb, zinc sulphate 1.25 lb, Pan Brtannica Co., plus 10 fl. oz. Manoxol wetter OT/A/60 gal).
7. Fernide, 4 lb (thiram, Plant Protection Co.)
8. Bayer 3114, 2 lb (dichofluanid, Baywood Chemical Co.)

9 & 10. Unsprayed control plots.

Weed control : Linuron and paraquat applied before bulbs emerged at makers recommended rates.

Fertilisers : None.

Cultural treatments : Primaries removed on appearance and one replaced in a central position in each plot in early March (Fig. 12B). Fungicidal sprays applied fortnightly at 60 gal/acre commencing mid-March.

Records : 1. Bulb weights at harvest.

2. Weekly leaf disease assessments on tagged plants.

RESULTS

Leaf disease assessments

Seasonal totals of disease integrals

Spray treatment	Replicate			Mean
	1	2	3	
Karamate	236	200	212	216
Orthocide and Murphane Wettable	204	256	250	239
Murphane Wettable	214	248	314	259
Liromate	280	288	312	293
Bayer 3114	296	270	332	299
Polyram	338	478	328	381
Tank-mix zineb	496	426	400	441
Fernide	680	534	494	569
Unsprayed controls	720	611	661	664

L.s.d. ($p = 0.05$) 112.7

($p = 0.01$) 154.8

Plot yields (lbs)

Spray treatments	Replicate 1	2	3	Mean
Karamate	19.4	22.2	21.0	20.8
Orthocide and Murphane Wettable	18.7	19.8	18.5	18.7
Murphane Wettable	17.8	20.0	17.8	18.5
Liromate	15.8	19.0	20.0	18.3
Bayer 3114	18.0	19.0	17.5	18.2
Polyram	16.4	16.6	17.8	16.9
Tank-mix zineb	14.1	14.9	15.2	14.7
Fernide	15.0	17.2	17.2	16.5
Unsprayed controls	11.9	13.3	13.4	12.9
			L.s.d. ($p = 0.05$)	1.50
			($p = 0.01$)	2.10

Regression data of yield on leaf disease integrals

Coefficient $b_{yx} = -0.13 \pm 0.003$

Equation $y = 151.8 - 0.13x$

where y = yield in lbs and

x = leaf disease integral.

Rosewarne E.H.S. 1966/67

Replicate blocks								
	I	6	8	3	5	2	4	1	7
	II	5	1	3	7	4	6	8	2
	III	7	4	5	1	6	8	2	3

Cultivar : Krelage Triumph

Grade : 8/9, 9/10 and 11/12 cm bulbs.

Design : 3 blocks each of 8 randomised plots. 200 bulbs, weighing 5.94 lbs/plot planted in 2 x 12 ft long rows each 2 ft apart, during October.

Experimental treatments : (weight of fungicide/60 gal).

1. Dithane 945, 2.5 lb (mancozeb, Pan Br tannica Ltd.)
2. Murphane Wettable, 2.5 lb (zineb, Murphy Chemical Co.)
3. Karamate, 3 lb (mancozeb with zineb, Pan Britannica Co.)
4. Tank-mix zineb (13.3 gm Dithane A 40, 19.4 gm zinc sulphate, 18.2 ml 'Redomite' petroleum oil/gal.)
5. Elvaron, 3 lb (dichlofluanid, Baywood Chemical Co.)
6. Elvaron, 2 lb.
7. Unsprayed controls.
8. Unlike treatments 1-7, plots in this treatment were freed from infectors and sprayed weekly with Dithane 945, 2.5 lb.

Weed control : Linuron and paraquat applied before bulb emergence at makers recommended rates.

Fertilisers : None.

Cultural treatments : Primaries removed on appearance and one replaced in a central position in each plot in early March (Fig. 12A). Fungicidal sprays fortnightly commencing mid-March at 60/galacre.

Records : 1. Bulb weights at harvest.

2. Weekly leaf disease assessments on tagged plants.

RESULTS

Plot yields (lbs)

Spray treatment	Replicate 1	2	3	Mean value
Murphane Wettable	15.2	14.8	13.8	14.7
Dithane 945	15.7	14.3	13.8	14.6
Karamate	15.1	13.8	13.5	14.2
'Healthy control'	14.2	14.2	13.1	13.9
Elvaron, 3 lb.	12.8	12.5	13.5	13.0
Elvaron, 2 lb.	13.8	12.6	12.5	13.0
Tank-mix zineb	12.8	12.7	12.9	12.8
Unsprayed control	11.8	11.6	13.5	12.3

L.s.d. ($p = 0.05$) 1.22

($p = 0.01$) 1.70

Kirton E.H.S. 1966/67

Replicate blocks					
I	2	3	1	6	8
	4	7	9	10	5
II	5	1	8	3	4
	10	7	6	2	9
III	6	3	2	1	9
	10	5	4	7	8

Cultivar : William Pitt

Grade : Row 1 - 10/11 cm and maiden bulbs, Ros 2-5 - 12/13 cm bulbs.

Design : 3 randomised blocks each of 10 plots. 200 bulbs, weighing 13.60 lbs planted in beds in 5 rows, each 8 in apart, in October.

Experimental treatments : (weights of fungicide/60 gal.)

1. Dithane 945, 2.5 lb (mancozeb, Pan Britannica Co.).
2. Murphane Wettable, 2.5 lb (zineb, Murphy Chemical Co.).
3. Karamate, 3 lb (mancozeb with zineb, Pan Britannica Co.).
4. Polyram, 2 lb (metiram, Boots Pure Drug Co.).
5. Tank-mix zineb (13.3 gm Dithane A 40, 9.4 gm zinc sulphate, 18.2 ml 'Redomite' petroleum oil/gal.).
6. Elvaron, 3 lb (dichlofluanid, Baywood Chemical Co.).
7. Elvaron, 2 lb.
8. Liromate, 4 lb (ferbam, Ligtermoet Chemie N.V.).
9. Unsprayed control.
10. Unlike treatments 1 - 9, plots in this treatment were freed from infectors and sprayed weekly with Dithane 945.

Weed control : Linuron and paraquat applied before bulb emergence at makers recommended rates.

Fertilisers : None.

Cultural treatments : Primaries removed on appearance and one replaced in a central position in each plot in early March (Fig. 12B). Fungicidal sprays applied fortnightly at 60 gal/acre commencing mid-March.

Records : 1. Bulb weights at harvest.

2. Weekly leaf disease assessments on tagged plants.

RESULTS

Leaf disease assessments

Seasonal totals of leaf disease integrals

Spray treatment	Replicate 1	2	3	Mean value
'Healthy control'	142	146	132	140
Dithane 945	198	192	194	194
Karamate	210	224	186	206
Elvaron, 3 lb	238	212	180	210
Murphane Wettable	220	220	264	234
Elvaron, 2 lb	294	252	280	242
Liromate	310	258	274	280
Tank-mix zineb	272	334	324	310
Polyram	336	334	274	314
Unsprayed control	496	502	534	517

L.s.d. ($p = 0.05$) 43.2

($p = 0.01$) 59.2

Plot yields (lbs)

Spray treatment	Replicate 1	2	3	Mean value
'Healthy control'	30.5	31.7	32.4	31.5
Dithane 945	29.2	32.5	33.3	31.6
Karamate	31.2	25.9	34.8	30.6
Elvaron, 3 lb	30.7	30.6	33.0	31.4
Murphane Wettable	29.3	25.8	22.7	25.9
Elvaron, 2 lb	27.8	33.2	30.0	30.3
Liromate	27.3	29.6	28.3	28.4
Tank-mix zineb	33.2	24.4	24.7	27.4
Polyram	24.6	22.9	28.5	25.2
Unsprayed control	19.3	17.0	21.0	19.1
			L.s.d. ($p = 0.05$)	5.1
			($p = 0.01$)	7.0

Regression data of yield on leaf disease integral

coefficient $byx = -0.26 \pm 0.097$

equation $y = 176.6 - 0.26x$

where y = yield in lb.

x = leaf disease integral

Appendix 10

Simulation of the losses caused by tulip fire by leaf clipping, 1966/67.

B	C	B	C	B	A	A	A	B	C	A	C
64	40	12	12	40	12	0	64	40	12	12	0
A	C	A	C	C	B	C	B	B	A	A	B
12	64	40	64	40	0	40	12	0	40	0	40
A	A	C	A	A	B	C	A	A	B	B	A
0	64	0	40	0	12	12	40	64	12	64	64
B	C	B	A	B	C	C	B	A	B	C	C
0	12	40	64	64	0	64	0	12	0	64	40

I II III IV

Replicate blocks

Cultivar : Rose Copland

Grade : 10/11 cm.

Planting and Lifting : 4 replicate blocks, each of 12 plots. 5 rows spaced 9 in apart within each plot, each row consisting of 10 bulbs in 'Netlon' 5 in apart. On 20th April, 18th May and 13th June one of the middle rows was sampled, being washed, roots and shoots removed, dried and weighed.

Treatments : Factorial combination of

1. Leaf pruning. Leaves cut on 26th March (A)
   Leaves cut on 17th April (B)
   Leaves cut on 15th May (C)

2. and differing proportion of leaf removed 0%

12%

40%

64%

RESULTS

Plot yield (gm dry wt) on 20th April of tulips leaf pruned on 26th March.

Leaf area decreases	Replicate 1	2	3	4	Total
No decrease	86.8	87.4	90.7	57.9	324.6
12% decrease	72.9	92.1	75.6	92.6	333.2
40% decrease	66.0	71.2	75.9	88.2	301.2
64% decrease	62.7	61.4	67.8	66.6	258.5

no significant difference

Plot yield (gm dry wt) on 18th May of tulips leaf pruned on (A) 26th March and (B) 17th April.

(A)					
No decrease	132.6	130.2	144.4	138.8	549.6
12% decrease	104.2	153.2	135.6	143.5	536.5
40% decrease	114.2	126.6	82.4	98.3	421.5
64% decrease	98.8	97.0	112.3	107.9	416.0
(B)					
No decrease	140.3	143.6	137.3	129.2	550.4
12% decrease	132.8	151.1	107.0	145.1	537.0
40% decrease	130.4	121.3	121.7	110.6	484.0
64% decrease	99.3	121.4	116.4	123.7	460.8

Plot yields (gm dry wt) on 13th June of tulips leaf pruned on (A) 26th March, (B) 17th April and (C) 15th May.

Leaf area decrease	Replicate 1	2	3	4	Total
(A)					
No decrease	138.5	151.5	175.9	207.1	673.0
12% decrease	153.4	163.8	185.6	135.5	638.3
40% decrease	83.6	129.7	146.7	154.8	514.8
64% decrease	131.9	120.6	140.0	112.3	504.8
(B)					
No decrease	132.4	141.0	189.0	138.7	601.1
12% decrease	192.6	164.6	154.2	157.9	669.3
40% decrease	177.2	152.0	128.9	124.0	582.1
64% decrease	116.6	132.6	84.8	115.2	449.2
(C)					
No decrease	188.5	139.6	135.0	192.3	655.4
12% decrease	131.7	177.8	186.9	159.6	656.0
40% decrease	163.7	149.8	153.8	111.9	579.2
64% decrease	173.4	101.7	159.7	156.3	591.1

Simulation, by leaf clipping, of losses caused by tulip fire, 1967/68

0	3	2	1
1	0	3	2
2	1	0	3
3	3	1	0

X

0	0	1	1	2	2	3	3
0	0	1	1	2	2	3	3
0	0	1	1	2	2	3	3
0	0	1	1	2	2	3	3

Y

0	0	0	1	1	1	2	2	2	3	3	3
0	0	0	1	1	1	2	2	2	3	3	3
0	0	0	1	1	1	2	2	2	3	3	3
0	0	0	1	1	1	2	2	2	3	3	3

Z

Cultivar : Rose Copland

Grade : 10/11 cm.

Design : 3 groups of plots, X, Y and Z, sampled in April, May and June respectively. Leaf pruning done (1) in March, (2) in April and (3) in May respectively. (0) unpruned plots.

40 bulbs/plot in 4 lots of 10, 5 in apart in 'Netlon', planted in 4 single ridges 24 in apart, in October.

Records : At each lifting, bulbs washed, daughter bulbs removed, dried at 80°C and weighed.

RESULTS

Plot yield (gm dry wt) in April of tulips leaf pruned in March.

Leaf area decreases	Replicate 1	2	3	4	Total
No decrease	31.3	31.8	30.5	33.2	126.8
12% decrease	29.8	31.7	33.6	32.5	127.6
40% decrease	25.2	27.8	24.5	29.3	106.8
64% decrease	16.1	29.4	22.7	20.6	88.8

Plot yield (gm dry wt) in May of tulips leaf pruned in (A) March and (B) April.

(A)					
No decrease	188.2	166.8	184.6	182.3	721.9
12% decrease	178.5	186.3	175.6	168.6	709.0
40% decrease	168.7	159.4	183.6	175.9	687.6
64% decrease	144.6	151.8	142.7	137.6	576.7
(B)					
No decrease	188.2	166.8	184.6	182.3	721.9
12% decrease	152.8	177.6	187.7	188.2	706.3
40% decrease	143.8	151.9	169.4	184.3	649.4
64% decrease	143.7	157.5	166.8	154.1	622.1

Plot yields (gm dry wt) in June of tulips leaf pruned in (A) March (B) April and (C) May.

Leaf area decreases	Replicate				Total
(A)	1	2	3	4	
No decrease	239.2	189.5	228.0	210.2	866.9
12% decrease	230.8	204.9	236.7	244.5	916.9
40% decrease	167.4	168.8	170.3	198.0	704.5
64% decrease	128.4	112.7	110.6	114.1	465.8
(B)					
No decrease	239.2	189.5	228.0	210.2	866.9
12% decrease	206.0	208.4	235.6	205.8	855.8
40% decrease	191.3	165.3	161.7	146.0	664.3
64% decrease	131.1	134.5	118.8	128.2	512.6
(C)					
No decrease	239.2	189.5	228.0	210.2	866.9
12% decrease	138.8	243.3	198.3	241.6	867.0
40% decrease	192.2	168.7	192.5	190.1	743.5
64% decrease	184.7	185.5	161.2	188.1	719.5

GLOSSARY

Aggressive foliar lesions, in contrast to non-aggressive lesions, progressively enlarge and ultimately bear sporulating colonies of B. tulipae.

'Blindstoken', a dutch term referring to the technique in which flowers are destroyed when bulbs are heated at c. 33°C for c. 4 weeks during storage. In the absence of flowers bulb yields are usually increased.

Darwin Hybrids form one of the twenty recognised groups of tulips. They are hybrids of Darwin tulips (Group 6) and Tulipa fosteriana Hoog. (Group 18), having long stems and large flowers which open earlier than Darwin tulip cultivars.

Field beds. Before widespread mechanisation, tulips were planted in single plough drawn furrows; every 7th or 8th remaining unplanted and serving as a path.

Forcing bulbs are subjected during storage to cool and warm temperature regimes so accelerating the formation of primordia and subsequent flowering.

Grade. Bulbs are sorted by size based upon their circumference. 10/11 cm bulbs are widely used for forcing; larger and smaller ones commonly being planted in the field.

Heading. At flowering tulips planted in the field are inspected for trueness to type and freedom from virus infections, subsequently being decapitated = headed. The removal of flowers enables metabolites to be diverted to the production of increased bulb yields.

Increase. Although sold commercially, the yields of bulbs at harvest are expressed as percentages of weights planted

earlier, the difference being increase which can be positive or negative.

Lifting. Ridge planted tulips are lifted and thrown behind using modified potato lifting machines, bulbs subsequently being collected manually.

Maiden bulbs are small bulbs which do not develop flowers. They usually consist of one or two fleshy scales, with the outer scale producing a single broad leaf.

Mother bulbs are those planted out of doors in autumn, and they decline as daughter bulbs enlarge in the axils of the scales.

Neck, the region of bulbs through which shoots emerge during winter.

Non-aggressive lesions caused by B. tulipae during spring and summer remain restricted and although B. tulipae remains viable it does not sporulate. The lesions appear as brown/white spots with black margins.

Primaries. This term refers to infected bulbs from which infected shoots develop and emerge above ground, and on which B. tulipae sporulates profusely to provide a source of inocula for secondary foliar infection.

Ridge planting is done with tractor mounted implements that draw out furrows, which are split back after planting. There are usually two rows of bulbs/ridge.

Rogueing. From bulb emergence in early spring, diseased and off-type bulbs are eradicated.

Root plate. The base part of bulbs, bearing adventitious roots beneath and fleshy scales and axillary buds above.

Tunic. The outer scale of bulbs which is initially white and fleshy gradually browning and becoming membraneous.

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